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The syngeneic mixed lymphocyte reaction in autoimmune susceptible mice

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THE SYNGENEIC MIXED LYMPHOCYTE REACTION in AUTOIMMUNE SUSCEPTIBLE MICE

by

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DISSERTATION

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Dedicated to

My mother and my father

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ABBREVIATIONS

AMLR = autologous mixed lymphocyte reaction

BGG = bovine gamma globulin

B/W = (New Zealand Black/New Zealand White) F_1

CML = cell-mediated lympholysis

Con A = Concanavalin A

cpm = counts per minute

DNP = dinitrophenyl

DNP-BGG = dinitrophenylated bovine gamma globulin

GVH = graft-versus-host

Hepes = N-2-hydroxyethyl piperazine-N-2-ethane sulfonic acid

HGG = human gamma globulin

HS = horse serum

IgM^+ IgM-bearing

LAF = lymphocyte activated factor

LPS = lipopolysaccharaide

MLC = allogeneic mixed lymphocyte culture

MLR = allogeneic mixed lymphocyte reaction

NA = non-adherent

NZ = New Zealand

NZB = New Zealand Black

NZW = New Zealand White

OS = obese strain

PBS = phosphate buffered saline

PHA = phytohemagglutinin

PNA = peanut agglutinin

PNA^+ = agglutinated by PNA

PNA^- = not agglutinated by PNA

SBA = soy bean agglutinin

SLE = systemic lupus erythematosus

SMLR = syngeneic mixed lymphocyte reaction

SRBC = sheep red blood cells

SS = Sjögren's syndrome

TCGF = T cell growth factor

$Thy1^+$ = Thy1-bearing

TNP = trinitrophenyl

^3H-TdR = tritiated thymidine

ABSTRACT

THE SYNGENEIC MIXED LYMPHOCYTE REACTION IN AUTOIMMUNE SUSCEPTIBLE MICE

In this study, the syngeneic mixed lymphocyte reaction (SMLR) was examined in four autoimmune mouse strains to search for a common immunological alteration shared by autoimmune-susceptible mice. The SMLR represents a proliferative response by splenic T-enriched cells to unfractionated syngeneic spleen cells that had been treated with mitomycin-C. The results presented in this dissertation indicate that all mouse strains genetically susceptible to the development of autoimmune disease had diminished SMLR compared to normal strains. This decreased SMLR may relate to disease severity, since it was most pronounced in autoimmune mice which expressed a severe form of the disease. For example, in B/W mice, the SMLR was lower at 7 months than at 2 months of age. It was also lower in BXSB males which develop more accelerated autoimmune disease than BXSB females. In addition, the SMLR was lower in MRL/l mice than in the related MRL/n mice which develop milder disease.

Reciprocal cell-mixing experiments were performed to determine the cell responsible for the reduced SMLR present in autoimmune mice. Results from such cell-mixing experiments suggested that the defect in these autoimmune strains resided within the responding population, whereas, the stimulating population appeared to behave normally. For example, it was observed that both young and old B/W unfractionated splenic stimulating cells were capable of provoking a response in young B/W T-enriched cells. By contrast, old B/W T-enriched responding cells failed to be

stimulated by young B/W splenic cells.

Furthermore, in B/W mice, a suppressor activity within the responding population did not appear to contribute to the reduced SMLR. Tne addition of old B/W responding cells to the young B/W responding cell population did not inhibit the SMLR of the latter. Moreover, in vitro exposure of responding cells with low-dose X-irradiation did not enhance the SMLR of B/W mice, suggesting the absence of an X-ray sensitive suppressor cell which might be inhibiting the activity of the responding cell in SMLR.

The decreased SMLR suggests the presence of a common T cell alteration shared by these several autoimmune strains or the presence of differing abnormalities whose common expression is through a selective T cell dysfunction. Therefore, characterization of the T-responding cell responsible for SMLR in normal mice was carried out in order to better define the nature of the defective cell contributing to the lowered SMLR present in autoimmune mice. Specific T cell subset surface markers and various physical properties possessed by the SMLR-responding cells were examined. It was demonstrated that the cell mediating SMLR beared the Lytl.2 surface antigen. Studies on the effects of several chemical manipulations on SMLR also indicated that the SMLR-responding cells were sensitive to the effects of X-irradiation, cyclophosphamide, and corticosteroid. Furthermore, experiments employing peanut agglutinin for fractionation of splenic T cells into two subpopulations suggested that the responding population of SMLR may be comprised of two subsets of T cells.

INTRODUCTION

The following section of the Introduction serves to orient the reader with my current understanding of the various concepts of cellular immunology. The roles of several cell types and their subpopulations in immune responses will be discussed. In addition, the various cellular events, cell-cell interactions and biologically active factors associated with immune reactions will be examined. It is hoped that these introductory remarks will provide the reader with the essential immunologic principles upon which most of the interpretations of the results presented in this dissertation is based.

I. CELLULAR PRINCIPLES OF THE IMMUNE SYSTEM

A. Participation by a Variety of Cells.

The immune system serves as a surveillance mechanism by which vertebrates are protected against pathogenic organisms and cancer cells. Through the activities of two major defenses within the immune system, humoral immunity and cellular immune reactions, foreign invaders are selectively eliminated. The humoral immune response basically resists bacterial and viral infections, whereas the cellular immune response mainly defends against intracellular viral infections, fungi, parasites, malignant cells, and foreign tissues.

Various cell types participate in immunity. Lymphocytes represent the major cells involved in immune responses. The presence of membrane receptors enables lymphocytes to specifically recognize and selectively interact with the foreign agent. With appropriate lymphocyte-antigen interactions, lymphocytes are activated and mediate various effector functions to eliminate foreign invaders.

The lymphocyte population is comprised of three main groups: B cells, T cells and null cells. B cells are responsible for the humoral aspects of immunity. When B cells are activated by foreign agents they differentiate to become plasma cells which secrete antibodies that specifically bind to the foreign invaders. Subsequently, a variety of elimination activities are triggered. T cells are the mediators of regulatory functions and cellular immune responses. Unlike B cells, T cells do not produce large amounts of antigen-binding soluble substances. T cells, however, appear to mediate their functions through direct cell-cell contacts or the release of factors which adhere to the surfaces of other cells. The third class of lymphocytes are the null cells which lack characteristics of both mature T and B cells (Stobo and Paul, 1973). Cytolysis appears to be the prominent activity of null cells (Kiessling et al, 1976).

Non-lymphoid cells also play important roles in immunity. These cells perform a wide variety of effector functions which enable effective elimination of foreign substances. Through the process of phagocytosis, macrophages serve as highly active participants of cellular immune reactions. In addition, macrophages participate both in cellular immunity reactions and humoral immune responses as auxillary cells during immune induction. Other cell types which include polymorphonuclear neutrophils, eosinophils, basophils and mast cells are involved in inflammatory reactions mediated by antibodies.

B. T Cells Mediate Various Functions.

T lymphocytes are capable of mediating a variety of effector functions. They can function as regulatory cells which influence B cell and other T cell activities. Acting as positive regulatory cells,

T cells cooperate with B cells to produce antibodies (Claman et al, 1966; Davies et al, 1967; Miller and Mitchell, 1967). Helper T cells also facilitate cytotoxic events mediated by killer T cells (Cantor and Boyse, (1975b). Suppressor T cells which negatively modulate immune activities specifically inhibit antibody production (Gershon and Kondo, 1970; Gershon and Paul, 1971; Basten et al, 1975; Warren and Davie, 1977) or T cell mediated functions (Gershon et al, 1972; Asherson and Zembala, 1974; Claman et al, 1974; Peavy and Pierce, 1974; Phanuphak et al, 1974; Rich and Rich, 1975; Rich and Rich, 1976). T cells are also able to react against cells bearing different histocompatibility antigens and thereby represent the primary cells responsible for allograft rejection. In addition, T lymphocytes possess the capacity to kill other cells and hence act as cytotoxic T cells. Other functions of cellular immunity mediated by T cells include delayed hypersensitivity, contact sensitivity, (Katz, 1977a), and resistance to certain bacterial infection.

Experiments utilizing anti-Lyt antisera have established that several distinct T cell subsets exist which are capable of mediating different effector functions. Lyt antigens are surface antigenic markers expressed only on thymus-derived cells (Cantor and Boyse, 1975a). The Lyt system of T lymphocytes is comprised of three different antigens, Lyt1, Lyt2, and Lyt3.* By employing specific antisera directed towards Lyt antigens, three T cell subpopulations have been defined: $Lyt1^+$, $Lyt23^-$ ($Lyt1^+$ cells), $Lyt1^-$, $Lyt23^+$ ($lyt23^-$ cells) and $Lyt1^+$, $Lyt23^+$ ($Lyt123^+$ cells (Cantor and Boyse, 1975a). These distinct subpopulations

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* Footnote: Scheid and Triglia (1979) recently demonstrated that T cells also bear the surface Lyt5. However, Lyt5 alloantigen is expressed on other cells of hematopoietic lineage such as prothymocytes, B cells, cells of myeloid and monocyte-macrophage types and natural killer cells.

appear to be distributed in varying proportions within the spleen. Janeway (1980) has reported the following proportion of Lyt subpopulations for splenic T cells: 45% $Lyt1^+$ cells, 14% $Lyt23^+$ cells and 35% $Lyt123^+$ cells. In addition, each of the subpopulations manifest different unique immune functions. $Lyt1^+$ cells have been demonstrated to mediate helper activities in antibody responses (Cantor and Boyse, 1975a), delayed type hypersensitivity reactions (Huber et al, 1976), and in vitro proliferative response to protein antigens (Janeway, 1980). Furthermore, $Lyt1^+$ cells are capable of responding to I-region differences in alloreactive proliferative responses (Cantor and Boyse, 1975a). Cytotoxic activities (Cantor and Boyse, 1975a) and suppressor functions (Cantor and Boyse, 1975a) are assigned to the $Lyt23^+$ cells. The function mediated by $Lyt123^+$ cells has not been clearly established, since it is difficult to isolate these cells in pure form. However, some experimental evidence suggests that $Lyt123^+$ cells may be precursors of $Lyt23^+$ killer cells in response to TNP-modified syngeneic cells (Cantor and Boyse, 1976). Hence, the Lyt system has enabled the elucidation of at least three distinct T cell subsets possessing unique immunological functions.

C. Cellular Interactions of Immune Response.

Immunity is a consequence of various complex cell-cell interactions. Several types of interactions between B cells and T cells are involved in the induction of antibody response to a large class of antigens which are thymus-dependent. One such interaction involving cooperation between B and T cells is characterized by three basic features. First, studies utilizing hapten-carrier conjugates as antigens have established that cooperation between B and T cells required an antigen

possessing two different antigenic determinants (Rajewsky et al, 1969; Mitchison, 1971). These experiments demonstrated that in order to obtain maximal anti-hapten response, T cells specific for the carrier determinants need to interact with B cells recognizing the hapten determinants. Furthermore, in these studies, it was observed that the determinants which stimulate helper T cells and B cells are required to be present on the same molecule for effective T-B collaboration. Secondly, accessory cells are necessary for optimal T-B cooperation. It appears that accessory cells such as macrophages are capable of presenting antigen to T helper cells and hence specifically lead to T cell priming (Mosier and Coppleson, 1968; Unanue, 1972; Pierce et al, 1974; Erb and Feldmann, 1975a; Erb and Feldmann, 1975b; Erb and Feldmann, 1975c). Experiments using guinea pig (Rosenthal, 1978) and mice (Erb and Feldmann, 1975d; Pierce et al, 1976) have indicated genetic identity in the I-region and not the K and D regions of the major histocompatibility complex is required for such T helper cell-macrophage cooperation. Thus, it appears that T helper cells recognize antigens in relationship with histocompatibility Ia products on the surface of macrophages. Thirdly, experiments in which the secondary anti-DNP response to DNP-BGG was analyzed also demonstrated that identity at the I-region is necessary for effective interaction between carrier-specific helper T cells and hapten-specific B cells (Benacerraf and Unanue, 1979a). Therefore, similar to macrophage-T cell interaction, I-region genes seem to restrict T-B cell collaborative interaction. From such findings, it appears that that the presentation of antigens by macrophages, involving Ia molecules on the surface of macrophages, determines the type of restriction associated with T-B cooperative interaction.

As in T helper-cell B cell interaction, the cellular interaction involving suppressor T cells is equally complex. It is not known whether T suppressor cells interact with other lymphocytes through cell surface components or through soluble factors. Various experimental studies have suggested that suppressor T cells may act directly on B cells (Basten et al, 1975; Warren and Davie, 1977), or affect carrier specific T helper cells (Tada, 1975). If they act on lymphocytes, it then appears that T suppressor cells could recognize cell-bound antigens or idiotypic determinants of T or B receptors. Suppressor factors have also been described (Takemori and Tada, 1975; Kapp et al, 1976).

Lyt123$^+$ T cells have recently been demonstrated to be important in the cellular interaction associated with regulation of immune response. The findings of Eardley et al (1978) suggest that Lyt123$^+$ lymphocytes possess bidirectional regulatory abilities. It was observed that when Lyt123$^+$ cells were added to an immune response which is moderate or at a high level, Lyt123$^+$ cells tend to act on Lyt23$^+$ suppressor cells resulting in suppressor activity. Addition of Lyt123$^+$ cells to weak immune response, however, tends to act on Lyt1$^+$ helper cells producing enhanced immune response. Thus, two opposite feedback loops were speculated to operate in immunoregulation: (1) in one set of feedback loop (feedback suppression), Lyt1$^+$ helper cells communicate with Lyt123$^+$ to produce Lyt23$^+$ suppressor cells; (2) in the other set of opposite feedback loop (feedback help), Lyt23$^+$ suppressor cells act on Lyt123$^+$ to induce more Lyt1$^+$ helper cells. Tada (1977) has also proposed a model in which Lyt123$^+$ cells appear to play an important and amplifying role in both helper and suppressor positive feedback loops. The structures recognized by Lyt123$^+$ cells are unknown, but major histocompati-

bility complex antigens and idiotypes are possible candidates.

Similar cellular interaction involved in the humoral immune response also occurs in cellular immunity. It is now well established that there is T-T cooperation in cell-mediated response in which one T cell subpopulation mediates effector function, while another acts as a helper cell. Asofsky et al (1971) were the first to demonstrate synergistic effects by two different T cell subpopulations in graft-versus-host (GVH) reaction. Their findings indicated that the injection of parental thymocytes and peripheral blood lymphocytes into F_1 hosts produced a spleen index in GVH which was greater than the sum of responses of each of the cell types alone. Five years later, by employing anti-Lyt antisera, this T-T cooperation in cell-mediated response was confirmed. In the studies by Cantor and Boyse (1975a), it was observed that $Lyt1^+$ cells alone were not capable of generating cell-mediated lympholysis (CML). $Lyt23^+$ cells alone were able to mediate CML, but killing represented only 30% of the control value. Mixtures of $Lyt1^+$ cells and $Lyt2^+$ cells, however, were able to generate the maximal level of cytolysis. Thus, two different T cell subpopulations interacted synergistically in CML responses. Further studies demonstrated that the $Lyt23^+$ cell served as an effector cell of cytolysis, while the $Lyt1^+$ cell acted as a helper cell (Cantor and Boyse, 1975b).

The dichotomy of antigen recognition by T helper cells and T effectors in cell-mediated response is also similar to that manifested by T helper cells and effectors in antibody production. As previously mentioned, in the induction of antibody formation, T helper cells recognize the carrier determinants, while B cells react with hapten determinants. Similarly, the three-cell experiment of Bach et al (1976)

suggests that in the generation of CML, the cytotoxic $Lyt23^+$ effector cells respond preferentially to K and D antigens on the stimulating cells, whereas the $Lyt1^+$ helper cells react preferentially to I-region antigens.

In conclusion, recent studies on the cellular aspects of immunity have generated the concept that both humoral as well as cellular immune responses are associated with a complex and intricate system of cellular interactions.

D. Participation of Biologically Active Factors in Immune Response.

Various biologically active factors derived from T lymphocytes or macrophages have been identified and characterized in order to study the mechanism of cell-cell interactions involved in immune response. Certain of these factors have been demonstrated to be antigen-specific, others non-specific, and also capable of mediating various immune activities. Most of the early investigations on these factors have dealt with T cell products which act on B cell functions. The antigen-specific T cell products which affect B cell activities include the immunoglobulin-like specific factors described by Feldmann et al (Feldmann, 1972; Feldmann and Basten, 1972a; Feldmann and Basten, 1972b; Feldman and Basten, 1972c; Feldmann et al, 1973) and Taniguichi and Tada (1974), the non-immunoglobulin specific factors possessing suppressive properties (reviewed in Tada, 1974, 1975a, 1975b; Kapp et al, 1976), and the non-immunoglobulin factors with enhancing activities (reviewed in Mozes, 1976). A variety of non-specific factors induced by antigens which are capable of stimulating B lymphocytes has also been reported (Hartmann, 1970; Rosenthal et al, 1973: Doria et al, 1972; Kishimoto and Ishizaka, 1973; Kishimoto and Ishizaka, 1974; Kishimoto

and Ishizaka, 1975). Furthermore, a number of investigators have obtained soluble factors from supernatants of cultures containing either histo-incompatible mouse spleens (Dutton et al, 1971; Andersson et al, 1972; Britton, 1972; Dutton and Hunter, 1974; Gorczynski et al, 1973; Schimpl and Wecker, 1972; Sjöberg et al, 1972; Watson, 1973), or mitogen-stimulated lymphocytes (Grey et al, 1971; Grey and Waksman, 1972; Rich and Pierce, 1974; Watson et al, 1973), which exert various biological effects in vitro. In addition to the T cell products which act on B cell activities, several non-specific factors affecting T cell responses have been reported (Solliday-Rich and Rich, 1975; Solliday-Rich and Rich, 1976; Altman and Cohen, 1975; Plate, 1976). One such non-specific T cell factor which has been fairly well-defined in terms of its biochemical and biological properties is T cell growth factor (TCGF).

TCGF is a product of T cells which have been stimulated with mitogens such as Concanavalin A (Con A) or alloantigens (Gillis et al, 1978; Larsson and Coutinho, 1979), whereas spleen cells stimulated with the B cell mitogen, lipopolysaccharide (LPS) are unable to generate TCGF (Andersson et al, 1979). The synthesis of TCGF by activated T cells depends upon the presence of splenic accessory cells which appear to possess surface Ia determinants (Larsson and Coutinho, 1979). Moreover, a macrophage product termed "lymphocyte activated factor" or LAF which is capable of promoting TCGF production by T cells has been reported. Smith et al (1979a) have demonstrated that splenic T cells stimulated with either phytohemagglutinin (PHA) or LAF did not produce TCGF. Restoration of TCGF synthesis, however, occurred when spleen cells were exposed to both PHA and LAF. Similar findings were also obtained by Larsson and coworkers (1980). Thus, two different signals are probably required

for TCGF production by T cells: (1) membrane binding by either mitogen or antigen; and (2) LAF.

TCGF with a molecular weight of 30,000 and comprising of two components which differ by charge (Watson et al, 1979) functions mainly as an inducer of T cell proliferation (Gillis et al, 1978; Watson et al, 1979; Smith et al, 1979b; Smith, 1980; Smith et al, 1980). This growth factor acts specifically on activated T cells but does not simulate resting T cells (Coutinho et al, 1980; Gillis et al, 1979). Moreover, TCGF does not act on lymphocytes which have been stimulated with the B cell mitogen, LPS (Coutinho et al, 1980). T cells which are activated by either mitogen or alloantigen and therefore become reactive to TCGF also express membrane receptors specific for TCGF. It was observed that these activated T cells are capable of absorbing TCGF, whereas unstimulated T cells do not (Smith et al, 1979b; Coutinho et al, 1980). Furthermore, the same TCGF responsive cells are not capable of producing TCGF (Andersson et al, 1979).

In conclusion, the above findings suggest that proliferation and differentiation in T cells are associated with several intricate interactions between cell types involving the participation of biologically active factors. Two major events appear to occur during the activation of T cell proliferation. First, a particular T cell subclass binds mitogen or antigen. Such mitogen/antigen membrane binding may bring about an interaction between this particular T cell subset with an Ia^+ accessory cell (proposed by Andersson et al, 1979). This interaction can lead to the formation of LAF by accessory cells. LAF then acts on the mitogen/antigen activated T cell subclass and further promotes the production of TCGF by these activated T cells. Secondly, a different class of T cells

also binds mitogen/antigen; as a result of this mitogen/antigen binding, these cells become responsive to TCGF.

II. AUTOIMMUNITY

A. General Concepts of Autoimmunity.

The immune system is able to specifically recognize an antigen and elicit a specific humoral or cellular response against the particular antigen. Furthermore, the immune system is capable of recognizing what is a foreign, "non-self" antigen and what is not foreign, a "self" antigen. The failure to discriminate between "self" and "non-self" antigens can lead to immune reactions towards "self" components; this immune response to "self" antigens is known as "autoimmunity". Autoimmune responses can be manifested by autoantibodies and/or by cellular immune reactions against "self" components.

Under normal conditions, the immune system does not produce any humoral or cellular response to "self" antigens. This inability to respond to "self" constituents has proven to be a puzzling and yet interesting problem for many immunologists. As early as 1959, Burnet (1959) in his clonal elimination hypothesis, proposed that "self" reactive clones are produced at an early stage in fetal life and then eliminated after contact with the "self" antigen. Thus, "self" reactive clones are never developed and do not exist in adults. This hypothesis was partly confirmed by the experimental studies of Billingham et al (1953). Their findings indicated that tolerance towards histocompatibility antigens could be induced following injections of allogeneic cells into new-born mice. Two recent observations, however, have weakened the "clonal elimination" concept of limiting autoimmunity. One such observation consists

of the ease in which autoimmune reactions can be induced in normal animals when they are exposed to altered forms of the autoantigen (Weigle, 1965). Secondly, normal human lymphocytes have been demonstrated to possess the capacity to bind autologous antigens (Bankhurst et al, 1973). Thus, the ability to recognize "self" antigens is present in adult life.

Two theories, therefore, were formulated to account for the absence of autoimmunity, despite the persistence of autoreactive clones. According to the first theory, tolerance against "self" components serves to limit autoimmunity. In the second theory, it is proposed that a mechanism involving suppressor T cells exists for preventing autoimmune responses (Allison, 1971; Weigle, 1971).

The first theory derives experimental support from studies on tolerance, the state of immunological unresponsiveness, to various foreign proteins. According to this hypothesis, three different types of tolerance states towards "self" constituents appear to be operating to prevent autoimmunity. These tolerant states against "self" antigens will first be discussed below. Following, experimental evidence to support the second theory which involves suppressor T cells will be presented.

For a certain class of "self" antigens, referred to as hidden autologous antigens, a state of no tolerance exists. These "self" constituents appear to be hidden from immune cells. Thus, tolerance against these "self" antigens is poorly developed, but immune response against these "self" components is easily induced. Because such "self" antigens are inaccessible to immune cells, autoimmune reactions towards these antigens usually do not occur. Immunity against hidden "self" antigens, however, readily occurs when they are exposed to the immune system. For example, autoantibodies directed towards heart proteins appear in serum

of patients with ischemic heart injury (Benacerraf and Unanue, 1979b). Hence, although tolerance against hidden "self" antigens is lacking, autoreactivity does not normally develop due to the inaccessibility of these "self" components to the immune system.

In another kind of tolerant state, low-dose or T cell tolerance exists for autoantigens which are present in trace amounts in blood or extracellular fluids. Under normal conditions, when the autologous antigen, thyroglobulin, is present in the blood at low concentration, T cell tolerance to thyroglobulin exists. T lymphocytes are actually unresponsive to thyroglobulin, but B lymphocytes are capable of responding to autologous thyroglobulin. Since there is a requirement for T-B cooperation for optimal immune response, B lymphocytes will not normally be activated to respond to thryoglobulin and produce autoantibodies against thyroglobulin. T cell tolerance towards such "self" antigen, however, can be bypassed by immunization with heterologous antigens. For example, Weigle and his collaborators (Nakamura and Weigle, 1967; Weigle and Nakamura, 1967) have demonstrated that autoallergic thyroiditis can be induced in rabbits upon immunization with heterologous thyroglobulin. In addition, such immunizations lead to the stimulation of B cell reactive to autologous thyroglobulin and thus autoantibodies directed towards "self" thyroglobulin. Weigle postulated that the introduction of foreign determinants in the autologous proteins such as cross-reactive determinants results in the stimulation of T lymphocytes recognizing this particular cross-reactive determinant. This stimulated T lymphocyte, in turn, is capable of providing help to B cells reactive to autologous thyroglobulin.

In a third type of tolerant state, tolerance at both B and T cell levels occurs with "self" antigens which are circulating in high concentrations. Autoimmune reactions towards serum proteins such as serum albumin have never been observed. Thus, it appears that high concentrations of "self" components in the serum maintain a very tolerant state. Both T and B lymphocytes have been shown to be non-reactive towards "self" components present in high concentration. Bankhurst et al (1973) further demonstrated that normal human B lymphocytes are capable of binding I^{125} human thyroglobulin, whereas no lymphocytes bind to human albumin.

The finding that suppressor T cells are able to negatively regulate immune response has led to extensive investigations on the role of suppressor T cells in tolerance induction. Some studies have suggested that suppressor T cells are involved in the maintenance of tolerance in certain experimental models, whereas such suppressor activity could not be demonstrated in other models (reviewed in Gershon, 1974; and Weigle, 1975). Likewise, for the same tolerogen such as human gamma globulin (HGG) some investigators were able to demonstrate T suppressor activity during tolerance (Basten et al, 1975; Benjamin, 1975; Doyle et al, 1976), while others failed to observe such suppression (Chiller et al, 1974; Zolla and Naor, 1974). At the moment, most immunologists working on tolerance tend to agree that T suppressor activity is not obligatory for the maintenance of tolerance, but suppressive activity can be generated during tolerance induction. Such T suppressor activity, however, is capable of contributing to the induction of an effective tolerant state.

It was further predicted that suppressor T cells may play an important role in the maintenance of tolerance against "self" antigens. Hence, it was not unexpected to find the emergence of a theory suggesting that suppressor T cells can serve to prevent autoimmunity in the presence of self-reactive cells. Based on the observation that low-dose or T cell tolerance towards "self" antigens can be easily bypassed by various events, a mechanism involving suppressor T cells for controlling autoimmunity was postulated by Allison et al (1971). The early experiments of Allison and his colleagues on New Zealand Black (NZB) mice, which spontaneously develop autoimmune hemolytic anemia, presented evidence that T lymphocytes are able to inhibit autoimmune reactions and further suggested that suppressor T cells may provide a general mechanism for delaying or preventing autoimmune response.

Several observations further provided evidence for the role of T cell mediated suppression in the control of autoimmunity. Morse et al (1974) have shown that congenitally thymus-deprived mice readily develop autoimmune manifestations. Likewise, mice postnatally deprived of their thymus possess higher incidences of autoantibodies (Yunis et al, 1967). Thymectomy increases the incidence of anti-nuclear factors in the obese strain (OS) and normal White Leghorn chickens (Albini and Wick, 1973). In rats, depletion of T lymphocytes also results in the spontaneous development of autoimmune thyroiditis (Penhale et al, 1973). These observations argue in support of the theory that T lymphocytes normally regulate the autoimmune process.

A more detailed analysis on the role of the thymus in controlling autoimmune thyroiditis was reported by Wick et al (1970). It was observed that B cell depletion by bursectomy abolished or markedly reduced

autoimmune thyroiditis which is spontaneously developed in OS White Leghorn chickens. In contrast, thymectomy of the OS White Leghorn chickens resulted in the worsening of the autoimmune process. In another study, mice genetically susceptible to autoimmune disease were shown to have accelerated disease when thymectomized (East et al, 1967; Talal, 1976). Furthermore, in the NZB/NZW F_1 (B/W) mice, which spontaneously develop autoimmune lupus-like disorders, multiple thymus grafts appeared to prolong their survival (Kysela and Steinberg, 1974). These results suggest that thymectomy probably eliminated suppressor T cells which serve to limit the autoimmune process.

Both defective T cell tolerance and T cell suppressor activities have been described in several murine strains which are genetically susceptible to autoimmunity. The autoimmune mouse strains, NZB and B/W, manifest abnormal tolerance induction with sheep red blood cells (SRBC) and deaggregated bovine gamma globulin (BGG) (Jacobs et al, 1971; Staples and Talal, 1969). Also, the suppressor T cell functions of NZB and B/W mice have been shown to be diminished in a variety of experimental systems (Dauphinee and Talal, 1973; Hardin et al, 1973; Barthold et al, 1974; Gerber et al, 1974; Dauphinee and Talal, 1975; Krakauer et al, 1976; Cantor et al, 1978). Thus, Talal (1976) speculated that the abnormal tolerance induction of NZB and B/W mice may reflect the loss of suppressor T cells.

In conclusion, autoimmunity can be considered as a breakdown of the regulatory system responsible for preventing or delaying autoimmune diseases. Most autoimmune disorders appear to involve immune responses towards "self" antigens which are present in low amounts in blood or extracellular fluids. Thus, low-dose or T cell tolerance to

these "self" antigens may provide a mechanism by which autoimmune reactions are limited. A loss of T cell tolerance towards such "self" components can lead to the emergence of "autoreactive" clones. The activation of T cells capable of providing help for response against "self" may bring about the loss of T cell tolerance to "self" antigens. If the expression of such T helper self-reactive activity is inhibited by suppressor T cells, then autoimmunity does not occur. In this case, a defect in T cell suppressor activity, however, would lead to autoimmune response. Alternatively, an abnormal T cell suppressor function can cause a loss of T cell tolerance towards "self" antigens. As a consequence, the emergence of self-reactive T helper cells or B cells occurs leading to the development of humoral and/or cell-mediated immune reactions against "self" antigens.

B. Autoimmunity in Man.

Humans manifest a wide spectrum of autoimmune diseases. At one end of the spectrum of autoimmunity reaction is the "organ-specific" disease associated with the presence of immune response directed towards "self" components of one specific organ (Spry, 1975). "Non-organ-specific" diseases in which immune reactivities are not confined to one organ constitute the other end of the spectrum (Spry, 1975). The "organ-specific" diseases include autoimmune endocrine disorders and autoimmune hemolytic anemia. An example of autoimmune endocrine diseases is Hashimoto's thyroiditis. In this disease, there are specific lesions in the thyroid involving infiltration of monocytes and destruction of the organ with the production of circulating antibodies against thyroid proteins such as thyroglobulin. Systemic lupus erythematosus (SLE), rheumatoid arthritis, Sjögren's syndrome (SS) and

rheumatic fever are examples of "non-organ-specific" autoimmune diseases. In SLE, pathological changes are widespread and primary lesions involve the connective tissues, skin or kidney. Patients with SLE also produce antibodies to native DNA, single-stranded DNA, a variety of nucleoproteins and cytoplasmic components. In general, autoantibodies with different specificities frequently occur in patients with autoimmune diseases (Glass and Schur, 1977).

C. Experimental Models for Autoimmunity.

Two different types of experimental models have been employed for studying autoimmunity. One involves the induction of autoimmune diseases by immunization of animals with autologous antigens in adjuvants or modified preparations of "self" antigens (Weigle, 1977). Using this type of immunization protocol, autoimmune reactions against a variety of "self" constituents such as sperm, thyroglobulin, adrenal, pancreas, and lens tissue have been investigated. In these studies, it appears that the normal regulatory mechanism responsible for preventing autoimmune diseases is overcome by such immunization processes. At the moment, it is not known whether the spontaneous autoimmune diseases in man are initiated in a similar way. Thus, this type of experimental model may not fully represent an accurate picture of the naturally occurring autoimmune diseases in man. The discovery of animals which spontaneously develop autoimmune disorders provided immunologists with a second type of experimental model for studying autoimmunity. Several animal models now exist for human autoimmune diseases. Spontaneous autoimmune thyroiditis occurs in OS White Leghorn chickens; therefore, these chickens serve as a model for Hashimoto's thyroiditis in man (Witebsky et al, 1969). The best known and extensively studied murine model for

autoimmunity is the NZB/NZW (B/W) mouse, a hybrid of the NZB and New Zealand White (NZW) strains. This strain spontaneously develops an autoimmune disease similar to human SLE (Burnet and Holmes, 1965; Mellor, 1966; Howie and Helyer, 1968). Two newly-developed mouse strains, BXSB and MRL/1, also serve as experimental models for SLE.

D. Murine Models for Human SLE.

The B/W, BXSB, and MRL/1 mice are now widely used as models for studying autoimmune SLE. As mentioned previously, these mice spontaneously develop a disorder resembling human SLE. The lupus-like disease in B/W, BXSB, and MRL/1 mice has been shown to be clinically and immunopathologically similar (Andrews et al, 1978; Murphy and Roths, 1978). B cell hyperactivity, autoantibodies to DNA, circulating immune complexes, immunoglobulin and complement abnormalities and severe glomerulonephritis are the predominant features in all of these strains.

Certain differences in the expression of the lupus-like disease exist among these mice. The age of onset and rapidity of progress of the disease in the three strains vary in that the BXSB and MRL/1 mice express their disease earlier and have more rapidly progressive disease than B/W mice (Andrews et al, 1978). Furthermore, there is an accelerated development of the disorder in B/W females (Howie and Helyer, 1968; Papoian et al, 1977) and BXSB males, but the disease is equally expressed in MRL/1 females and males (Andrews et al, 1978). In the B/W, the disease appears in females at 6 months of age associated with a 50% mortality at 8-1/2 months of age, while the males manifest the disorder later in life, with a 50% mortality at about 15 months of age (Roubinian et al, 1977). By the third and fourth month of age, BXSB males begin

to show signs of the disease manifesting a 50% mortality at 5 months of age. The disease occurs late in BXSB females, and at 15 months of age, there is a 50% mortality. The MRL/l also expresses its disease earlier, and by 5 months of age, a 50% mortality is present. The related strain, MRL/n, however, develops autoimmunity much later in life (Murphy and Roths, 1978).

Differences in serological abnormalities are also observed for the various autoimmune strains (Andrews et al, 1978). The Ig levels, which correlate best with the severity of the disease in the several strains are higher in BXSB and MRL/l mice than B/W mice. Anti-nucleic acid antibodies, h owever, are elevated in all mice with the highest amounts being present in the B/W and modest levels in BXSB and MRL/l. In general, the amount of circulating immune complexes are higher in MRL/l mice than in BXSB and B/W mice.

A lymphoproliferative disorder is present in the newly-developed BXSB and MRL/l mouse strains (Murphy and Roths, 1978) but not in the B/W strain. At 2 months of age, all MRL/l mice develop massive generalized lymph node enlargement, while the MRL/n mice do not develop the lymphoproliferative disorder. T lymphocytes appear to be the predominant cell types of the lymphoproliferation. The BXSB mice, however, develop moderate lymphadenopathy and splenomegaly in which lymphocytes of B cell origin are the major cell types present.

E. Immune Functions of Mice Susceptible to SLE.

Extensive immunologic investigations on these autoimmune strains have been performed in hopes of finding a common functional defect that might bring insight into the pathogenesis of autoimmunity. There is an abundance of evidence to suggest that the immune systems

of NZB and B/W mice are functioning aberrantly. Abnormalities of B cells, T cells and macrophages (McCombs et al, 1975) have been described. Because of the complex nature of the immune system, the determination of which cell is responsible for the abnormal immune activities present in autoimmune mice is becoming more difficult to resolve. As a result, contradictory findings concerning the nature of the defective cell or cells present in these strains of mice are reported. For example, some studies found deficient T cell functions in NZB and B/W mice, while other failed to report such abnormalities. Thus, such analyses of immune functions in these autoimmune mice have not fully resolved the question of what abnormalities of the immune system are related to the autoimmune process.

For the remaining part of this Introduction, I wish to present an overall view on the immune status of the various autoimmune strains, particularly the New Zealand (NZ) strains.

Immunological abnormalities are detected at a very young age in NZ mice. Adult levels of antibody responses are reached within the first week of life (Cerottini et al, 1969). Compared to normal mice, young NZB and B/W mice produce excessive amounts of antibodies to several selected antigens such as foreign proteins, SRBC, and synthetic nucleic acids (Playfair, 1968; Weir et al, 1968; Staples and Talal, 1969; Steinberg et al, 1969). In addition, newborn NZB splenic cells produce increased quantitites of IgM and demonstrate a polyclonal hyper-responsiveness compared to age-matched conventional mouse strains (Cohen et al, 1978). Enhanced cell-mediated functions also appear early in NZ mice. For example, young NZ mice are capable of regressing tumors more rapidly than age-matched control strains (Gazdar et al, 1971).

Hence, the NZ strains seem to develop immunologic competence prematurely.

Young NZB and B/W mice appear to be relatively resistant to the induction and maintenance of immunologic tolerance to deaggregated HGG and BGG (Staples and Talal, 1969; Staples et al, 1970). By 2 months of age, these mice cannot be made tolerant to BGG. Up to 3 weeks of age, tolerance, however, can be induced in NZB and B/W mice, but this tolerance is rapidly lost. Staples and Talal speculated that a defective T cell tolerance may be responsible for the early escape from tolerance to BGG in the very young NZ mice. Furthermore, abnormalities present in either T or B cells may account for the inability of these autoimmune mice to maintain tolerance to BGG.

The generalized immunologic hyperactivity and relative resistance to immunologic tolerance present in NZ mice may reflect an impaired T cell regulation. As discussed earlier, at least two T cell subpopulations are involved in the regulation of immune response. Helper T cells serve to positively regulate immune activities, whereas suppressor T cells negatively modulate immune functions. A variety of experimental studies have demonstrated that NZB and B/W mice possess increased helper T cell functions but decreased suppressor T cell activities.

Suppressor T cell activities have been shown to decrease between 1-2 months of age in NZB mice as demonstrated in GVH assays (Dauphinee and Talal, 1973; Hardin et al, 1973), in the response to pneumococcal polysaccharide (Barthold et al, 1974), and in the response to polyvinylpyrrolidine (Bach et al, 1977). This premature decline in suppressor function occurred at the same age as the loss of immunologic tolerance. Thus, it is possible that the resistance to immunological tolerance manifested by the NZB mice may reflect a loss in T suppressor functions.

Other studies, however, have indicated that young B/W mice possess normal suppressor activity, and as these mice age, the suppressor function declines. For example, Gerber et al (1974) have reported a loss with age in B/W mice of thymic suppressor cells using the GVH assay. In addition, Krakauer et al (1976) found that 1-month old B/W mice mediated normal suppressor activity, as determined by the ability of Con A-activated spleen cells from 1-month old B/W mice to inhibit pokeweed mitogen driven IgM biosynthesis in vitro. On the other hand, adult B/W mice (4-1/2 months of age) had reduced suppressor activity in that Con A-activated spleen cells from adult B/W mice were not able to inhibit pokeweed mitogen driven IgM biosynthesis in vitro. The above studies support the view that NZB and B/W mice begin to lose suppressor functions early in life, and that these suppressor activities further decline with age. This view has recently been challenged by Roder et al (1977), who demonstrated that suppressor cell activities of B/W mice increased rather than decreased as these mice aged. Primi et al (1978) also found that Con A-activated spleen cells from old NZB mice were able to inhibit the polyclonal response of young NZB and Balb/c spleen cells and concluded that the impaired suppressor activity of NZB mice was not related to a suppressor T cell deficiency.

In general, older NZB and B/W mice have marked impairment in various T cell functions. They manifest decreased response to mitogens (Leventhal and Talal, 1970) and to alloantigens (Falkoff et al, 1978), diminished ability to induce GVH disease and reject tumors (Cantor et al, 1970; Gelfand and Steinberg, 1973; Gershwin et al, 1974). A decline in long-lived recirculating lymphocytes and theta-positive cells within the lymph node and spleen is also apparent in older NZ mice (Talal, 1974).

The immunological abnormalities present in the older autoimmune mice, however, may partially be related to the aging process and to the spontaneous development of autoantibodies cytotoxic to thymocytes and T lymphocytes (Shirai and Mellors, 1971).

Some T cell functions, however, appear to be excessive at certain ages in NZB mice. Palmer et al (1976) have reported that the proliferative response to alloantigens is increased in NZB mice from 1 to 4 months of age. Similarly, recent work by Falkoff et al (1978) also indicated that NZB mice at 4 to 6 months of age display enhanced T cell helper activities as measured by proliferative response to PHA or alloreactive antigens; after 6 months of age, the PHA and alloantigen responses begin to decline. Increased helper T cells or decreased suppressor T cells may contribute to the hyperactive proliferative responses to mitogens and alloantigens observed in NZB mice. Most experimental assays do not distinguish between an increase in helper activity and a decrease in suppressor activity. Hence, the relative changes in helper and suppressor cell activities cannot be measured in these assays. Because of such experimental limitations, it has been difficult to resolve whether an increase in helper T cells or a decrease in suppressor cells is present in autoimmune NZB mice.

The Lyt antisera have revealed the existence of at least three distinct T cell subsets. Further analyses utilizing anti-Lyt antisera also support the concept that the regulation of immune responses depends upon immunological circuits involving complex interactions among different T cell subpopulations (Gershon, 1977). Recently, abnormalities within immunologic circuits were suggested for three autoimmune mouse strains, NZB, BXSB and MRL/l (Cantor and Gershon, 1979). In the NZB

strain, the major T cell lesion is an absence or malfunction of $Lyt1^{+}23^{+}$ T cells mediating feedback inhibition (Cantor et al, 1978). $Lyt1^{+}$ cells from MRL/1, however, are resistant to the suppressor signals produced by $Lyt1^{+}23^{+}$ feedback suppressor cells (Gershon et al, 1978). It then appears that the NZB, MRL/1 and BXSB strains share a common immunologic disorder: a defective communication among cells involved in regulation of immunity. These findings further suggest that the autoimmune process may be associated with a major impairment in the T cell regulatory network rather than a one-cell defect.

In addition to T cell abnormalities, some studies have reported defective B cell functions in the NZ mice. For example, Morton and Siegel (1974) have suggested that an abnormal hemopoietic stem cell contributes to the autoimmune disease in NZB mice. In their studies, they showed that bone-marrow derived cells of young NZB mice were capable of transmitting autoantibody functions to lethally irradiated histocompatible non-autoimmune mouse strains. The work of Primi et al (1978) also indicated that a B cell defect may be responsible for the impaired suppressor activity present in NZB mice. Furthermore, it was observed by Izui et al (1978) that the four autoimmune mouse strains, NZB, B/W, BXSB, and MRL/1 possessed enhanced polyclonal activation of B lymphocytes. The authors postulated that a primary B cell hyperactivity may contribute to the abnormal polyclonal activation of B lymphocytes in the autoimmune strains. Thus, these findings indicate that a common B cell defect is shared by the four autoimmune strains.

The recent work of Creighton et al (1979), however, failed to detect the presence of abnormalities involving either T or B cells in young or older autoimmune mice. By measuring the immune response to

well-defined hapten-carrier conjugates, they found that the regulatory T cells, both helper and suppressor cells, as well as B cells behaved normally in these autoimmune mice. These observations raise some doubts concerning the hypothesis that abnormalities of the immune system may be related to the autoimmune process and further contribute to the pathogenesis of autoimmunity. Despite the overwhelming number of studies which demonstrated various immune defects associated with autoimmune mouse strains, the findings of Creighton et al should not be ignored. Such contradictory findings are, indeed, unsettling, but further careful analyses of the immune status in these autoimmune mice are required before any definite conclusions on the role of a defective immune system in the pathogenesis of autoimmunity can be reached.

III. SYNGENEIC MIXED LYMPHOCYTE REACTION (SMLR)

The syngeneic mixed lymphocyte reaction (SMLR) represents a proliferative response when murine splenic T-enriched cells are stimulated with mitomycin-C treated unfractionated spleen cells (Ponzio et al, 1975; Smith and Pasternak, 1978). The responding cell in SMLR is a T cell, since anti-brain associated T cell antigens plus complement pretreatment of the responding population abolished the proliferative response (Smith and Pasternak, 1978). It also possesses a $Lyt1^+$, 23^-phenotype (Glimcher et al, 1980). The stimulating cell involved in SMLR has not been well-characterized, but non-T splenic cells are capable of stimulating SMLR. Both B cells and macrophages have been shown to stimulate syngeneic T cells. Von Boehmer (1974) has reported that B cells are the stimulating cells in SMLR. Finke et al (1976) also indicated that the IgG_1-bearing B cells are strong stimulators of SMLR. In contrast, Glimcher et al (1980) suggested that the stimulating cell population is a macrophage

possessing surface Ia antigens. Unlike the allogeneic mixed lymphocyte reaction (MLR), the peak response of SMLR occurs on day-5 of culture. In addition, it appears that no cytotoxic activity against syngeneic target is developed during SMLR (Smith and Pasternak, 1978).

A proliferative response similar to SMLR occurs in humans when T cells are exposed to autologous non-T cells (Opelz et al, 1975; Kuntz et al, 1976; Hausman and Stobo, 1979). This response is referred to as the autologous mixed lymphocyte reaction (AMLR). Various laboratories have demonstrated that the AMLR responding cells are enriched in cells which are capable of developing suppressor activity (Innes et al, 1979; Sakane and Green, 1979; Smith and Knowlton, 1979). Helper activity is also observed in the AMLR responding population (Chiorazzi et al, 1979; Hausman and Stobo, 1979). As in the mouse, there is a divergence of opinions concerning the nature of the cell which stimulates autologous human T lymphocytes. It is well established that the stimulating cell of AMLR is a non-T cell. Some laboratories have reported that the most effective stimulator is a B cell (Opelz et al, 1975; Smith, 1978), while others have indicated that the majority of stimulating activity is due to B cells and monocytes (Sakane et al, 1978b; Hausman and Stobo, 1979). One laboratory, however, demonstrated that most of the stimulating cells possessed characteristics of a K lymphocyte (Kuntz et al, 1976).

Recently, several laboratories have reported that patients with SLE have decreased AMLR (Sakane et al, 1978b; Kuntz et al, 1979; Sakane et al, 1979). Similarly, Smith and Pasternak (1978) also indicated that autoimmune NZB mice manifest poor SMLR compared to normal strains of mice.

In this thesis, the SMLR was employed to search for a common immunological alteration shared by autoimmune-susceptible mice. The SMLR was

examined in four autoimmune murine strains. All mouse strains genetically susceptible to the development of autoimmune disease had diminished SMLR compared to normal strains. This decreased SMLR may relate to disease severity. In addition, it appeared that a defect within the T responding cell population was responsible for the diminished SMLR. Therefore, further characterization of the SMLR-responding cell was performed in the hopes to better define the nature of the T cell defect involved in autoimmune mice.

MATERIALS AND METHODS

I. MICE

C57BL/6 and CBA/J mice were purchased from Jackson Laboratories, Bar Harbor, MN, and housed in the vivarium at the Veterans Administration Medical Center, San Francisco, CA. All autoimmune strains were bred and raised at the Univeristy of California Animal Care Facility, Hunters Point, San Francisco, CA. The starting NZB and NZW breeding colony originated from the National Institutes of Health, Bethesda, MD, and the first BXSB, MRL/l and MRL/n mice were provided by Jackson Laboratories, Bar Harbor, MN.

II. MEDIA

Media used for cell preparations consisted of RPMI-1640 (Cell Culture Facility, University of California, San Francisco) supplemented with 10mM Hepes (Sigma, St. Louis, MO), 1% antibiotic-antimycotic mixture, 100x (GIBCO, NY), 0.1 mg/ml gentamicin (Shearing Corp., Kenilworth, NJ), and 5% heat-inactivated horse serum (HS) (Kansas City Biological Inc., Lenexa, Kansas or GIBCO).

For culturing of cells, RPMI-1640 supplemented with 10mM Hepes, 1% L-glutamine, 200mM (GIBCO), 5×10^{-5} M 2-Mercaptoethanol, 1% antibiotic-antimycotic mixture and 10% heat-inactivated HS was used.

III. CELL PREPARATION

Spleen cell suspensions were processed according to the method described by Mishell and Dutton (1967). After washing once, the spleen cells were resuspended to approximately 1×10^{8} cells/ml and were then further fractionated using several different techniques:

A. Nylon Wool Fractionation.

Nylon wool columns were employed to obtain a T-enriched non-adherent (NA) cell population. They were prepared according to the procedures of Julius et al (1973), with the following modifications: (1) nylon wool was detoxified by boiling for 1 hour in distilled water, followed by soaking in a 37°C incubator with daily changes of water over a one-week period; and (2) media employed for nylon wool columns was that used for cell preparations described under Media.

Spleen cells were filtered through nylon wool in a similar manner as described by Julius et al (1973). T cell enrichment of the NA cell fraction from nylon wool columns was demonstrated by enhancement of proliferative response to Con A and PHA.

T cell depleted adherent cell population was recovered immediately after eluting the NA cell fraction. About 10 ml of media was added to the column; the nylon wool was then vigorously agitated with a stirring rod and the loosened cells were squeezed out of the nylon wool with a plunger. This step was repeated one more time.

B. Soy Bean Agglutinin (SBA) Separation of Spleen Cells.

Splenic cells were fractionated into B and T cells according to a modified procedure of Reisner et al (1976b). A single cell preparation of spleen cells ($2x10^8$ cells in 0.5 ml RPMI with 10mM Hepes) was incubated with SBA (0.5 ml of 2 mg/ml in PBS); (Vector Laboratories, Burlingame, CA) for 15 minutes at room temperature. The cells were then gently layered with a Pasteur pipette on top of 50% heat-inactivated HS (20 ml HS plus 20 ml RPMI with 10mM Hepes) in a 50 ml polystyrene conical tube. After incubating at room temperature for 30 minutes, the top and bottom fractions (approximately 10 ml) were separately removed with

Pasteur pipettes. To the 2 fractions, 20 ml of 0.2M D-galactose in PBS was added. The cell suspensions were centrifuged, resuspended with 15 ml of 0.2M D-galactose and then incubated at 37°C for 10 minutes. After this, the cells were washed twice with D-galactose followed by two additional washes with RPMI with 10 mM Hepes, supplemented with 5% HS. The top and bottom fractions constituted 11% and 40% of the recovered cells, respectively. Reisner et al (1976b) demonstrated that the unagglutinated top fraction was mainly comprised of theta-bearing cells and responded strongly to Con A and PHA and poorly to LPS. In addition, the agglutinated bottom fraction possessed a high percentage of IgG or IgM bearing cells which displayed poor responses to Con A and PHA and strong responses to LPS.

C. Peanut Agglutinin (PNA) Separation of Spleen Cells.

Spleen cells were separated into two subpopulations based on their differential agglutination by PNA (Reisner et al, 1976a; Roelants et al, 1979). A suspension of splenic cells ($3x10^8$ cells in 1.5 ml of RPMI, 10mM Hepes) was incubated with PNA (0.5 ml of 2 mg/ml, Vector Laboratories, Burlingame, CA) for 15 minutes at room temperature. At the end of the incubation period, the cells were gently layered with a Pasteur pipette on top of a 50% mixture of heat-inactivated HS and RPMI, 10mM Hepes. After incubating for 30 minutes at room temperature, the top and bottom fractions were removed separately. A solution of 0.2M 1-O-methyl-α-D-galactopyranoside in PBS was immediately added to the two separate fractions and centrifuged. The packed cells were then re-suspended with 15 ml of the 0.2M 1-O-methyl-α-D-galactopyranoside and incubated for 10 minutes at 37°C. Following the incubation, the fractions were washed twice with RPMI supplemented with 5% HS. The top fraction

comprised of cells which were not agglutinated by PNA, whereas the bottom fraction consisted of cells which were agglutinated by PNA.

D. Treatment with Anti-Thy1.2 plus Complement.

Monoclonal anti-Thy1.2 (Lot #LK-147) was purchased from (New England Nuclear, Boston, MA). The antiserum was titered against mouse thymus cells using lyophilized guinea pig complement (GIBCO) that had been reconstituted with 0.01 M PBS pH 7.2. Greater than 98% of the thymus cells were killed by the antiserum at 1:40,000 dilutions of the antiserum. To insure effective killing of $Thy1.2^+$ cells, 1:5,000 of anti-Thy1.2 was chosen as the working dilution.

In a total volume of 1 ml, $1x10^7$ NA cells from nylon wool fractionation were incubated with anti-Thy1.2 (1:5,000) for 30 minutes at 4^oC. The cell mixture was then washed once, and incubated with guinea pig complement (1:4) for 30 minutes at 37^oC in 5% CO_2 (Raff, 1971).

E. Treatment with Anti-Lyt Antigens plus Complement.

Rat monoclonal antibodies to the T cell surface antigens, Lyt1.2, Lyt2.2, and Lyt3 were kindly provided by Dr. Jeffrey Ledbetter, Department of Genetics, Stanford Medical Center, Stanford, CA). The specificity of the various anti-Lyt antiserum used in this study is described elsewhere (Ledbetter and Herzenberg, 1979). For selective killing of cells bearing the Lyt antigens, killing was performed using selective rabbit complement obtained from weanling rabbits. Before use, rabbit complement was tested for low toxicity against either thymocytes or spleen cells.

In a total volume of 1 ml, $1x10^7$ NA cells were incubated with either monoclonal anti-Lyt1.2 (1:80), anti-Lyt2.2 (1:50) or anti-Lyt3 (1:60) for 45 minutes at 4^oC. Following, the cells were washed once,

resuspended with 1 ml of mouse anti-rat immunoglobulin (1:50) (kindly provided by Dr. Jeffrey Ledbetter) and incubated for 30 minutes at 4°C. Following this incubation, the cell mixture was then centrifuged. One ml of an appropriate dilution of rabbit complement was then added to the cell pellets. After incubation for 30 minutes at 37°C in 5% CO_2, the cells were extensively washed 5X.

F. Treatment with Anti-IgM plus Complement.

Goat anti-IgM (Mu chain specific, Lot #129492) was purchased from Cappel Laboratories, Inc., Cochranville, PA. The antiserum was titered against normal spleen cells using rabbit complement obtained from weanling rabbits. A 1:10 dilution of anti-IgM (at 3 mg/ml) was determined as optimal. At this dilution of antiserum, the anti-IgM plus complement did not cause significant killing of thymocytes.

For the selective depletion of IgM-bearing (IgM^+) cells, $1x10^7$ NA cells were incubated with anti-IgM (1:10) for 45 minutes at 4°C in a total volume of 1 ml. The cell mixture was then washed and treated with rabbit complement (1:6) for 30 minutes at 37°C in 5% CO_2.

G. X-Irradiation.

NA cells,at a concentration of $1x10^7$ cells/ml, were exposed to various doses of irradiation from a Westinghouse Quadrocentex generator (Radiation Oncology, University of California, San Francisco).

H. Cyclophosphamide Treatment.

Mice were injected i.p. with cyclophosphamide (Cytoxan, Mead Johnson, Evansville, IN). For low-dose cyclophosphamide treatment, mice were injected with 20 mg cytoxan/kg of body weight. Mice treated with high-dose cyclophosphamide were given 100 mg cytoxan/kg of body weight (Katz, 1977b). Control mice were injected with PBS. The spleens of

the treated mice were then prepared for culturing 24 hours after the injection.

I. Hydrocortisone Treatment.

Various concentrations of hydrocortisone-21-sodium succinate (Sigma, St. Louis, MO) were added at the beginning of cocultures of NA cells and mitomycin-C treated unfractionated syngeneic splenic cells.

J. Depletion of Adherent Cells from Spleen via Petri Dishes.

For the depletion of adherent cells from the spleen, 5 ml of spleen cells ($1x10^7$ cells/ml) in fresh culture medium was added to petri dishes (15mmx50mm) and allowed to attach. After incubating for 2 hours at 37°C in 5% CO_2, non-adherent cells were gently removed with Pasteur pipettes and washed twice (Mosier, 1967). These non-adherent cells were then treated with mitomycin-C, and the ability of non-adherent splenic cells to stimulate SMLR was determined.

A non-adherent cell population was also obtained from spleen cells which were cultured overnight in petri dishes. For the culturing of splenic cells, between 5-6 ml of spleen cells ($5x10^6$/ml) in fresh culture medium was added to petri dishes (15mmx60mm). The cultures were incubated overnight at 37°C in 5% CO_2. Following the incubation, non-adherent cells were gently removed. These non-adherent cells from overnight cultures of splenic cells were then used as stimulators for the SMLR.

IV. CELL CULTURES for SMLR

The SMLR assay was performed according to the modified method of Smith and Pasternak (1978). NA cells from nylon wool fractionation were cocultured with mitomycin-C treated unfractionated syngeneic spleen cells (50 µg mitomycin-C/10^7 cells for 1 hour at 37°C). Triplicate or

quadruplicate assays were performed in 96-well culture plates (Linbro #76-205-05; Flow Laboratories, Ingelwood, CA), using 0.2 ml of cells/well. Between $8x10^5$ and $1.2x10^6$ responding cells, and between $2x10^5$ and $8x10^5$ stimulating cells/well were employed. Cultures were incubated in 5% CO_2 for 5 days, and pulsed with 10 μCi of H^3-thymidine (Schwarz/Mann, Orangeburg, NY) during the last 18-20 hours of culture. Cultures were then harvested onto glass filter papers (Reeve Angel, Grade 934 AH, Clifton, NJ) employing an Otto Hiller 12-channel filtration unit (Otto Hiller, Madison, WI). The filters were placed in polyethylene minivials (Packard Instruments, Burlingame, CA) containing Liquifluor-Toluene scintillation fluid and counted in a Packard liquid scintillation spectrometer. Results are presented in two ways: (1) net incorporation in cpm: cpm incorporated by stimulated cultures - cpm incorporated by unstimulated cultures (cpm of unstimulated cultures consist of cpm of responding cells alone + cpm of stimulating cells alone); and (2) stimulation index = cpm of stimulated cultures/cpm of unstimulated cultures.

V. CELL CULTURE for MLR

The allogeneic mixed lymphocyte reaction (MLR) assay was performed by a method similar to the SMLR assay with the following exceptions: (1) mitomycin-C treated unfractionated allogeneic spleen cells were employed as stimulating cells; and (2) cultures were incubated for 4 days.

RESULTS

I. THE SMLR in VARIOUS MOUSE STRAINS

The SMLR was examined in several murine strains. Varying concentrations of T-enriched responding cells were cocultured with several different concentrations of mitomycin-C treated syngeneic unfractionated splenic cells. The optimal cell concentration for the cultures of normal mice was $1x10^6$ responding and $4x10^5$ stimulating cells. Peak response in the SMLR occurred on day-5.

The magnitude of the SMLR varied in different strains, but mice genetically susceptible to autoimmune diseases had a lower SMLR than normal mice such as C57BL/6 and CBA/J (Table 1). Both female and male B/W mice and the two recently developed autoimmune strains, the BXSB and MRL/l, exhibited weak SMLR. In most experiments, no SMLR was detected in older B/W and BXSB mice. High incorporation of H^3-TdR was often observed in the unstimulated cultures of autoimmune mice. Hence, some cultures showed high delta cpm associated with a low stimulating index.

II. THE SMLR in AUTOIMMUNE MICE CORRELATED WITH DISEASE ACTIVITY

The decreased SMLR observed in mice genetically susceptible to autoimmune disease correlated with disease severity. The abilities of several groups of autoimmune mice manifesting different clinical stages of their disease to mediate SMLR were compared. In both female and male B/W mice, the SMLR was lower at 7 than at 2 months of age (Table 2). This difference in SMLR between younger and older B/W mice was even greater when employing higher concentration of responding cells. Seven-month olf B/W females, whose disease is more advanced than males, gave no response in SMLR. In contrast, the magnitude of SMLR for non-autoimmune

C57BL/6 mice was similar for young and old mice (Table 2). In fact, the SMLR in the C57BL/6 strain was somewhat increased with age when the stimulation index was compared. A similar finding was also reported for the CB17 mice (Smith and Pasternak, 1978).

The SMLR was more decreased in B/W females than in B/W males (Table 3). In contrast, it was lower in the BXSB males, in which disease is more accelerated in comparison to females (Table 3). In fact, the SMLR of BXSB females was strong compared to that of BXSB males. This difference in SMLR was even greater than that between female and male B/W mice.

In BXSB mice, as in the B/W mice, the SMLR declined with age (Table 4). The SMLR was weak in 3-month old BXSB males but absent in 6-month old BXSB males. This age-related difference in SMLR was not seen in the BXSB females which were not yet ill at the ages studied.

The SMLR was also lower in MRL/l mice than in the related MRL/n strain which develops milder autoimmune disease (Table 5). In one experiment no SMLR was detected in 2-month old MRL/l mice, and in another experiment a weak SMLR was observed. The experiments presented in Table 5 as well as others not reported here indicated that the SMLR of 2-month old MRL/n mice was somewhat variable, ranging from fairly weak to moderately strong response.

III. THE RESPONDING POPULATION BEHAVED ABNORMALLY in SMLR

Cell mixing experiments were performed to determine the cell responsible for the decreased SMLR present in autoimmune mice. The ability of T-enriched responding cells from young or old autoimmune mice to be stimulated by young or old syngeneic non-T cells was examined. Studies in both B/W and BXSB mice indicated that the stimulating population behaved normally and that the defect resided within the responding population.

In Table 6, both young and old B/W unfractionated splenic stimulating cells were capable of provoking a response in young B/W T-enriched cells. However, old B/W T-enriched responding cells failed to be stimulated by young B/W splenic cells. Similar findings were observed in young and old male BXSB mice (Table 7).

The difference between female and male BXSB mice provided another way to analyze the cellular basis for the decreased SMLR. As shown earlier, 2-month old BXSB females were capable of giving a strong SMLR, whereas 2-month old BXSB males gave a weak response. Therefore, the ability of female and male BXSB splenic cells to stimulate female BXSB T-enriched responding cells was tested. The results in Table 8 illustrate that female and male BXSB spleen cells stimulated BXSB female responding cells equally. Furthermore, the response of BXSB male T-enriched cells did not improve when exposed to BXSB female rather than male stimulating cells. It appears, then, that the decreased SMLR of BXSB male mice was due to a defect in the responding population which was also true of the B/W mice.

In the MRL/l mice, the defective cell type was also found within the responding population (Table 9). It was observed that both MRL/l and MRL/n spleen cells were equally capable of stimulating MRL/n responding cells. The SMLR of MRL/l responding cells, however, was not further enhanced when stimulated with MRL/n spleen cells.

An increase in suppressor activity within the responding population did not appear to be responsible for the lowered SMLR present in old B/W mice. The addition of two different concentrations of 7-month old B/W T-enriched cells to cocultures of 2-month old B/W T-enriched responding cells and syngeneic splenic stimulating cells did not cause a decrease

in SMLR (Table 10).

In addition, pretreatment of the responding cells with low-dose X-rays did not alter the SMLR of B/W mice (Tables 11, 12, 13), suggesting the absence of an X-ray sensitive suppressor cell which might be inhibiting the activity of responding cells in the SMLR. The results of Tables 11 and 12 indicated that pretreating 3-month old B/W responding cells with various low doses of irradiation did not enhance the SMLR. The SMLR, however, appeared to decline upon pretreating the responding population with low amounts of X-rays. Similarly, exposing 8-month old B/W responding cells to low-dose X-rays also did not cause an increase in SMLR (Table 13).

IV. HIGH BACKGROUND PROLIFERATION of BXSB MALE MICE

As previously mentioned, high incorporation of H^3-TdR was often observed in unstimulated cultures of autoimmune mice, particularly the BXSB females and males. One cause for the high background proliferation may be the presence of contaminating B cells or macrophages within the NA cell population from nylon wool fractionation. Since BXSB male mice have higher proportion of splenic B cells than non-autoimmune mice such as C57BL/6, it is possible that fractionation of BXSB spleen cells on nylon wool may give a less pure population of T-enriched cells than fractionation with normal spleen cells. In addition, most background proliferation is due to non-T cells such as B cells, macrophages or null cells. This is supported by the observation that the background proliferation of whole spleen cells was always reduced when these cells are passed through nylon wool, while most of the background proliferation was retained by cells adhering to nylon wool (unpublished observation). Therefore, in an attempt to better understand the nature of the high

background proliferation displayed by BXSB mice, the following three types of experiments were performed:

First, the percentage of IgM^+ cells and Thy1-bearing ($Thy1^+$) cells within NA cell population from nylon wool fractionation was compared between BXSB males and CBA males. In this study, the number of IgM^+ cells and $Thy1^+$ cells were enumerated by first treating NA cells with either anti-IgM or anti-Thy1.2. After incubating at 4^oC for 45 minutes, the cells were washed once and treated with rabbit complement. These cell mixtures were incubated for an additional 30 minutes at 37^oC in 5% CO_2 and then washed twice. The number of dead cells was determined by the trypan blue exclusion test. Results in Table 14 suggest that the BXSB male NA cell population contained fewer $Thy1^+$ cells and higher numbers of IgM^+ cells than the CBA male NA fractions. Thus, the decreased numbers of T cells and increased numbers of B cells within the NA cell fraction of BXSB males may be responsible for the high proliferation of unstimulated cultures.

Secondly, attempts were made to eliminate the cell type within the NA cell population of BXSB males which may contribute to the high background proliferation. NA cells from BXSB males were pretreated with anti-IgM plus rabbit complement and then cultured with mitomycin-C treated unfractionated syngeneic spleen cells. It was observed that pretreatment with anti-IgM plus complement did not reduce the proliferation of unstimulated BXSB male cultures (Table 15). Furthermore, anti-IgM and complement pretreatment did not appear to significantly alter the SMLR of BXSB males when compared to the complement control. Thus, eliminating IgM^+ cells within the NA fraction did not reduce the background proliferation of BXSB male mice.

Thirdly, the effects of pretreating NA cells from BXSB spleens with anti-Thyl.2 plus complement was also examined. Results in Table 16 indicated that the background proliferation in cultures containing only BXSB NA-responding cells was reduced by more than 60%. As expected, the proliferation of cocultures of BXSB NA-responding cells plus mitomycin-C treated unfractionated spleen cells was also reduced, since T cells are the mediators of SMLR.

V. ABSENCE of DIMINISHED RESPONSE to ALLOANTIGENS WITH AGE in B/W MICE

The proliferative responses in allogeneic lymphocyte culture (MLC) of 4-month and 8-month old B/W male mice were compared. As shown in Table 17, no difference in the ability to respond to alloantigens was detected for the 4-month and 8-month old B/W mice.

VI. CHARACTERIZATION of the RESPONDING CELLS MEDIATING SMLR

A. Responding Cells of SMLR Mainly Reside Within NA Population of Nylon Wool Fractionation.

The ability of the adherent cell population from nylon wool fractionation to mediate SMLR was also examined. The results in Table 18 indicated that the adherent fraction from CBA spleen cells responded poorly to syngeneic spleen cells compared to the NA fraction. In most experiments, it was also observed that the background proliferation of the adherent cell population was usually higher than that of the NA population. Hence, the majority of cells responding to syngeneic spleen cells was found within the NA population of nylon wool fractionation.

B. Soy Bean Agglutinin (SBA) Separations Indicated that T Cells Mediate SMLR.

Reisner et al (1976b) reported that under suitable conditions mouse splenic B cells were agglutinated by SBA, whereas T cells were not.

They demonstrated that the agglutinated cells could be separated from the non-agglutinated cells by gravity sedimentation. Furthermore, the agglutinated cells could be dissociated into single viable cells by D-galactose, a specific hapten inhibitor of SBA. Utilizing such a separation approach with SBA, C57BL/6 spleen lymphocytes were separated into two fractions: a non-agglutinating T cell population, and an agglutinating B cell population. Table 19 illustrates that the cells which responded strongly to syngeneic spleen cells were not agglutinated by SBA; splenic cells which responded weakly to syngeneic spleen cells, however, were agglutinated by SBA. By employing a cell separation technique based on differential agglutination by SBA, it was demonstrated that the responding cell of SMLR is a T cell.

C. The Responding Cell in the SMLR is a $Thy1^+$ Cell.

Treatment of the responding population with monoclonal anti-Thy1.2 plus guinea pig complement abolished most of the SMLR (Table 20), suggesting that the cell mediating SMLR is a T cell. Smith and Pasternak (1978) previously showed that treatment of the responding population with anti-brain associated T cell antigen plus complement eliminated the SMLR.

D. The Responding Cell in SMLR Bears the Lyt1.2 Surface Antigen.

Selective killing of NA cells bearing Lyt1 antigens indicated that the SMLR responding cells possess the $Lyt1^+$, 23^- phenotype. In Table 21, pretreatment of the NA population with anti-Lyt1.2 plus rabbit complement markedly diminished the SMLR; anti-Lyt2.2 plus complement, however, did not significantly alter the SMLR compared to untreated or complement controls.

E. Effect of Various Chemical Manipulations on the Responding T Cells in SMLR and MLR.

Prior to the establishment of the Lyt surface antigens, the distinction between various functional subpopulations of T cells was mainly based on their differential sensitivities to either thymectomy and anti-lymphocytic serum, X-irradiation, cyclophosphamide, or corticosteroids. Thus, in this study, three different kinds of chemical manipulations were employed to distinguish the responding T cell mediating SMLR from the responding cell responsible for the MLR. In the following subsection, the effect of various chemical manipulations on the responding T cells in SMLR and MLR will be considered.

1. X-Irradiation:

The effects of various low doses of X-rays on the SMLR and MLR in C57BL/6 mice were examined. NA cells were exposed to different amounts of X-irradiation, ranging from 50-200 rads and were then co-cultured with the appropriate mitomycin-C treated unfractionated splenic cells. These studies suggested that the responding cell of SMLR and that of MLR appeared to possess different sensitivities to X-irradiation. According to the "net incorporation" data in Table 22B, a marked decrease in the proliferative response to syngeneic splenic cells was observed with the 50 rads pretreatment. The "stimulation index" data, however, indicated that a fairly strong SMLR occurred with the 50 rads treatment, but that the SMLR was severely diminished at 200 rads. Such discrepancy is due to the fact that the background proliferation of the SMLR responding cell is quite sensitive to X-irradiation. For example, the results in Table 22B indicated that the proliferation of the unstimulated responding population in SMLR declined upon treatment with 50 rads of X-irradiation. Similarly, analyses of the total incorporation of H^3-TdR

by the stimulated cultures of SMLR suggested that a decrease in the proliferative response against syngeneic spleen cells occurred when the responding cells were exposed to 50 or more rads of X-irradiation. It appears then that the SMLR-responding cell or its precursor is very sensitive to X-irradiation. The responding cell in MLR, however, appeared to be more resistant to the effects of X-irradiation than the responding cell in SMLR. In Table 22A, the results demonstrated that the MLR was significantly lowered when the responding cells were exposed to at least 200 rads of X-irradiation. Furthermore, a decrease in the proliferation of the unstimulated cultures in MLR was already apparent at 50 rads, while at this similar dose of X-irradiation, the response in the stimulated cultures appeared not to be altered. (The proliferative response of the unstimulated cultures for MLR actually represents a SMLR on day-4.) Most likely, the responding cells which survived the exposure to 50 rads of X-rays (also to 100 rads) constituted the majority of T cells mediating MLR. In conclusion, pretreating NA responding cells with 50 or more rads of X-irradiation resulted in a diminished SMLR, whereas the MLR was not affected until the responding cells were exposed to 200 rads of X-rays. Similar findings on the effects of low-dose X-irradiation on SMLR and MLR were observed in B/W mice (Tables 23A and 23B).

2. Cyclophosphamide:

Administration of a single injection of high-dose (100 mg/kg) cyclophosphamide in C57BL/6 mice almost completely abolished the SMLR (Table 24A). Such in vivo pretreatment with high-dose cyclophosphamide, however, only resulted in a slight decrease in MLR (Table 24B). As in the X-irradiation experiments, the background proliferation of SMLR was also decreased upon in vivo treatment with high-dose cyclophosphamide.

In contrast, the administration of low-dose cyclophosphamide (20 mg/kg) did not alter the SMLR and MLR in C57BL/6 mice (Tables 25A and 25B).

3. Corticosteroids:

It is well known that corticosteroids are capable of suppressing various immune functions. In addition, T cell subpopulations appear to display different sensitivities to corticosteroids (Kass and Finland, 1953; Berglund, 1956; Segal et al, 1972). Therefore, in an attempt to further distinguish the SMLR-responding cell from the MLR-responding cell, the in vitro effects of hydrocortisone succinate on SMLR and MLR cultures were examined. Various concentrations of hydrocortisone were added to the initial period of either the SMLR or MLR cultures. It was observed that over 80% suppression of SMLR occurred with the addition of $8x10^{-8}$M hydrocortisone succinate (Table 26). In contrast, the presence of $8x10^{-8}$M hydrocortisone during MLR inhibited 15-20% of the proliferative response (Table 27).

F. Both PNA^+ and PNA^- T Cells Mediate SMLR and MLR.

Peanut agglutinin (PNA) has been employed for the fractionation of thymocytes into two subpopulations. Reisner et al (1976a) demonstrated that the major immature thymocyte subpopulation was readily agglutinated by PNA, while the minor mature subpopulation was not agglutinated with lectin. In addition, Roelants et al (1979) recently reported that a small proportion of splenic T cells was capable of binding PNA. They further demonstrated that the splenic cells agglutinated by PNA comprised almost equal numbers of $Thy1^+$, Ig^- and $Thy1^-$, Ig^- cells. Thus, it was interesting to determine whether cell separation studies using PNA can distinguish the responding cell of SMLR from that of MLR.

NA cells were incubated with PNA; cells agglutinated by PNA (PNA^+) and those which were not (PNA^-) were separated by gravity sedimentation. The agglutinated cells were further dissociated into single-viable cells with 1-0 methyl-α-D-galactopyranoside. The isolated PNA^+ and PNA^- subpopulations from NA cells were then cocultured with either mitomycin-C treated unfractionated syngeneic or allogeneic spleen cells. Table 28A illustrated that both C57BL/6 PNA^+ and PNA^- cells responded equally to allogeneic cells. Also, the proliferative response by PNA^+ cells towards syngeneic cells was similar to that by PNA^- subpopulations from 9-month old B/W male mice (Tables 29A and 29B).

VII. CHARACTERIZATION of STIMULATING CELLS in SMLR

A. Organ Distribution of Stimulating Cells.

Mouse syngeneic spleen cells are capable of stimulating T cells to mediate a strong proliferative response. The ability of cells from other lymphoid organs to stimulate SMLR was examined. It was observed that bone marrow cells and thymocytes were unable to provoke a SMLR (Table 30). Furthermore, cells obtained from lymph nodes stimulated a somewhat weak proliferative response by syngeneic T cells. Thus, it appears that the spleen provides the major source of cells capable of stimulating syngeneic T cells to undergo a strong proliferative response.

B. Physical Properties of Splenic Stimulating Cells.

The adherent subpopulation from nylon wool fractionation of spleen cells acts as strong stimulating cells for SMLR, whereas the non-adherent subpopulation from nylon wool serves as weak stimulators. Results in Table 31 indicated that the syngeneic proliferative response towards mitomycin-C treated adherent cells from CBA spleens was greater

than the response against unfractionated CBA spleen cells. In contrast, splenic nonadherent cells provoked a rather weak SMLR compared to whole spleen cells.

The splenic stimulating cells in SMLR also possessed binding capacities for SBA and PNA. Spleen cells which were agglutinated by SBA provoked a slightly stronger SMLR than unfractionated spleen cells (Table 32). Studies utilizing PNA demonstrated that both PNA^+ as well as PNA^- spleen cells stimulated syngeneic T cells to proliferate vigorously (Table 33). PNA^+ spleen cells, however, appeared to be more potent stimulators of SMLR than PNA^- splenic cells.

Eliminating the adherent population from spleen cells did not alter their capacity to stimulate syngeneic T cells. Spleen cells depleted of cells adhering to plastics were still quite capable of stimulating a strong SMLR compared to unfractionated spleen cells (Table 34). Such observations tend to suggest that spleen cells which do not readily adhere to plastics are strong stimulators of SMLR. But it is possible that incomplete depletion of adherent cells can contribute to the presence of the strong stimulatory effect. For the depletion of adherent cells, spleen cells were permitted to attach once onto the plastic petri dishes; after incubating for a short period of 2 hours, the nonadherent fraction was gently collected. It is probable that the nonadherent fraction can contain sufficient numbers of contaminating adherent cells which are capable of stimulating a strong SMLR.

Furthermore, the nonadherent population from spleen cells cultured overnight in petri dishes was shown to also strongly stimulate syngeneic T cells. In fact, the results in Table 35 indicated that the nonadherent fraction from cultured spleen cells was a better stimulator

of SMLR than fresh unfractionated spleen cells. The increased stimulatory effect manifested by these nonadherent cells may be due to membrane alterations occurring on the stimulating cells during the overnight culture. Such nonadherent cells from splenic cultures, however, stimulated allogeneic T cells almost as well as fresh spleen cells (Table 35).

TABLE 1 SMLR in Various Mouse Strains[a]

Strains[b]	Sex	Age (mos)	Incorporation of ^{3}H-TdR (cpm) Unstimulated	Stimulated	Net Incorporation	S.I.[d]
C57BL/6	F	2	3,262 (955)[c]	14,932 (1,712)	11,670	4.6
C57BL/6	F	6	1,126 (241)	14,952 (513)	13,826	13.3
C57BL/6	M	2	4,161 (1,749)	21,324 (1,410)	17,163	5.1
CBA/J	F	2	1,781 (133)	13,567 (3,746)	11,786	7.6
NZB	F	2	5,589 (46)	9,049 (790)	3,460	1.6
B/W	F	7	5,446 (105)	2,955 (27)	-2,491	-
B/W	M	7	2,467 (369)	3,356 (506)	889	1.4
BXSB	M	5	8,550 (593)	7,584 (578)	-966	-
MRL/1	M	2	4,080 (186)	6,009 (760)	1,929	1.5

[a] $1x10^6$ non-adherent T-enriched splenic responding cells were cocultured with $4x10^5$ mitomycin-C treated unfractionated syngeneic spleen cells for 5 days

[b] The SMLR of each mouse strain presented here represents a typical response from at least three experiments

[c] ( ) = Standard deviation

[d] S.I. = Stimulation index

TABLE 2 SMLR in 2-Month Old and 6-7 Month Old Mice

Responder[a]	Stimulator[b]	Incorporation of ^{3}H-TdR (cpm) Unstimulated	Stimulated	Net Incorporation	S.I.[d]
B/W Male Mice:					
$1x10^6$ 2 mos	$4x10^5$ 2 mos	1,693 (148)[c]	3,504 (676)	1,811	2.1
$1x10^6$ 2 mos	$8x10^5$ 2 mos	1,693 (148)	4,722 (942)	3,029	2.8
$1.2x10^6$ 2 mos	$4x10^5$ 2 mos	2,275 (292)	8,425 (57)	6,150	3.7
$1.2x10^6$ 2 mos	$8x10^5$ 2 mos	2,275 (292)	12,390 (1,047)	10,115	5.5
$1x10^6$ 7 mos	$4x10^5$ 7 mos	2,467 (369)	3,356 (506)	889	1.4
$1x10^6$ 7 mos	$8x10^5$ 7 mos	2,467 (369)	3,536 (186)	1,069	1.4
$1.2x10^6$ 7 mos	$4x10^5$ 7 mos	2,875 (16)	6,762 (984)	3,887	2.3
$1.2x10^6$ 7 mos	$8x10^5$ 7 mos	2,875 (16)	4,430 (70)	1,555	1.5
B/W Female Mice:					
$1x10^6$ 2 mos	$4x10^5$ 2 mos	1,867 (54)	2,461 (69)	594	1.3
$1x10^6$ 2 mos	$8x10^5$ 2 mos	1,867 (54)	3,826 (151)	1,959	2.0
$1.2x10^6$ 2 mos	$4x10^5$ 2 mos	2,729 (261)	4,663 (166)	1,934	1.7
$1.2x10^6$ 2 mos	$8x10^5$ 2 mos	2,729 (261)	6,148 (536)	3,419	2.3
$1x10^6$ 7 mos	$4x10^5$ 7 mos	5,446 (105)	2,955 (27)	-2,491	-
$1x10^6$ 7 mos	$8x10^5$ 7 mos	5,446 (105)	1,508 (279)	-3,938	-
$1.2x10^6$ 7 mos	$4x10^5$ 7 mos	4,814 (298)	3,628 (419)	-1,186	-
$1.2x10^6$ 7 mos	$8x10^5$ 7 mos	4,814 (298)	1,915 (37)	-2,899	-

-continued-

TABLE 2 (Continued) SMLR in 2-Month Old and 6-7 Month Old Mice

Responder[a]	Stimulator[b]	Incorporation of ^{3}H-TdR (cpm)		Net Incorporation	S.I.[d]
		Unstimulated	Stimulated		
C57BL/6 Female Mice:					
$1x10^6$ 2 mos	$4x10^5$ 2 mos	3,806 (212)[c]	14,098 (489)	10,292	3.7
$1x10^6$ 2 mos	$8x10^5$ 2 mos	3,806 (212)	18,459 (2,213)	14,653	4.8
$1x10^6$ 6 mos	$4x10^5$ 6 mos	2,115 (283)	16,321 (2,118)	14,206	7.7
$1x10^6$ 6 mos	$8x10^5$ 6 mos	2,115 (283)	17,195 (2,119)	15,080	8.1

[a] Non-adherent T-enriched splenic responding cells

[b] Mitomycin-C treated unfractionated syngeneic spleen cells

[c] ( ) = Standard deviation

[d] S.I. = Stimulation index

TABLE 3 SMLR in 2-Month Old Female and Male Mice

Responder[a]	Stimulator[b]	Incorporation of ^{3}H-TdR (cpm) Unstimulated	Stimulated	Net Incorporation	S.I.[d]
B/W Mice:					
$1x10^6$ female	$4x10^5$ female	6,097 (809)[c]	10,575 (497)	4,478	1.7
$1x10^6$ female	$8x10^5$ female	6,097 (809)	7,753 (1,784)	1,656	1.3
$1x10^6$ male	$4x10^5$ male	2,945 (261)	9,306 (2,202)	6,361	3.2
$1x10^6$ male	$8x10^5$ male	2,945 (261)	12,044 (43)	9,099	4.1
BXSB Mice:					
$1x10^6$ female	$4x10^5$ female	4,210 (1,384)	38,607 (3,759)	34,397	9.1
$1x10^6$ female	$8x10^5$ female	4,210 (1,384)	42,287 (88)	38,077	10.0
$1x10^6$ male	$4x10^5$ male	11,935 (170)	20,282 (259)	8,347	1.7
$1x10^6$ male	$8x10^5$ male	11,935 (170)	19,483 (392)	7,548	1.6

[a] Non-adherent T-enriched splenic responding cells

[b] Mitomycin-C treated unfractionated syngeneic spleen cells

[c] ( ) = Standard deviation

[d] S.I. = Stimulation index

TABLE 4 SMLR in 3-Month old and 6-Month Old BXSB Mice

Responder[a]	Stimulator[b]	Incorporation of ^{3}H-TdR (cpm) Unstimulated	Stimulated	Net Incorporation	S.I.[d]
BXSB Male Mice:					
$1x10^6$ 3 mos	$4x10^5$ 3 mos	11,485 (26)[c]	24,584 (2,463)	13,099	2.1
$1x10^6$ 3 mos	$8x10^5$ 3 mos	11,485 (26)	25,143 (276)	13,658	2.2
$1x10^6$ 6 mos	$4x10^5$ 6 mos	12,690 (297)	11,845 (31)	-845	-
$1x10^6$ 6 mos	$8x10^5$ 6 mos	12,690 (297)	10,288 (13)	-2,402	-
BXSB Female Mice:					
$1x10^6$ 3 mos	$4x10^5$ 3 mos	5,596 (1,270)	24,453 (511)	18,857	4.4
$1x10^6$ 3 mos	$8x10^5$ 3 mos	5,596 (1,270)	30,655 (131)	25,059	5.5
$1x10^6$ 6 mos	$4x10^5$ 6 mos	10,274 (791)	50,895 (660)	40,621	4.9
$1x10^6$ 6 mos	$8x10^5$ 6 mos	10,274 (791)	43,630 (2,627)	33,356	4.2

[a] Non-adherent T-enriched splenic responding cells

[b] Mitomycin-C treated unfractionated syngeneic spleen cells

[c] ( ) = Standard deviation

[d] S.I. = Stimulation index

TABLE 5 SMLR in 2-Month Old MRL/1 and MRL/n Substrains

Responder[a]	Stimulator[b]	Incorporation of ^{3}H-TdR (cpm) Unstimulated	Stimulated	Net Incorporation	S.I.[d]
Experiment #99:					
$1x10^6_6$ MRL/1	$4x10^5_5$ MRL/1	9,658 (1,117)[c]	6,989 (356)	-2,669	-
$1x10^6$ MRL/1	$8x10^5$ MRL/1	9,658 (1,117)	7,867 (582)	-1,791	-
$1x10^6_6$ MRL/n	$4x10^5_5$ MRL/n	10,648 (2,250)	24,528 (3,992)	13,880	2.3
$1x10^6$ MRL/n	$8x10^5$ MRL/n	10,648 (2,250)	17,493 (59)	6,845	1.6
Experiment #112:					
$1x10^6_6$ MRL/1	$4x10^5_5$ MRL/1	4,080 (186)	6,009 (760)	1,929	1.5
$1x10^6$ MRL/1	$8x10^5$ MRL/1	4,080 (186)	6,932 (1,352)	2,852	1.7
$1x10^6_6$ MRL/n	$4x10^5_5$ MRL/n	4,270 (6)	14,647 (2,594)	10,377	3.4
$1x10^6$ MRL/n	$8x10^5$ MRL/n	4,270 (6)	15,076 (362)	10,806	3.5

[a] Non-adherent T-enriched splenic responding cells

[b] Mitomycin-C treated unfractionated syngeneic spleen cells

[c] ( ) = Standard deviation

[d] S.I. = Stimulation index

TABLE 6 Failure of 2-Month Old Male B/W Splenic Cells to Stimulate 7-Month Old Male B/W Responding Cells

Responder[a]	Stimulator[b]	Incorporation of ^{3}H-TdR (cpm) Unstimulated	Stimulated	Net Incorporation	S.I.[d]
B/W Male Mice:					
$1x10^6$ 2 mos	$4x10^5$ 2 mos	1,693 (148)[c]	3,504 (676)	1,811	2.1
$1x10^6$ 2 mos	$8x10^5$ 2 mos	1,693 (148)	4,722 (942)	3,029	2.8
$1x10^6$ 2 mos	$4x10^5$ 7 mos	1,693 (148)	6,309 (2,433)	4,616	3.7
$1x10^6$ 2 mos	$8x10^5$ 7 mos	1,693 (148)	7,423 (2,742)	5,730	4.4
$1x10^6$ 7 mos	$4x10^5$ 2 mos	2,467 (369)	3,247 (1,163)	780	1.3
$1x10^6$ 7 mos	$8x10^5$ 2 mos	2,467 (369)	4,018 (257)	1,551	1.6
$1x10^6$ 7 mos	$4x10^5$ 7 mos	2,467 (369)	3,356 (506)	889	1.4
$1x10^6$ 7 mos	$8x10^5$ 7 mos	2,467 (369)	3,536 (180)	1,069	1.4

[a] Non-adherent T-enriched splenic responding cells

[b] Mitomycin-C treated unfractionated syngeneic spleen cells

[c] ( ) = Standard deviation

[d] S.I. = Stimulation index

TABLE 7 Failure of 2-Month Old Male BXSB Splenic Cells to Stimulate 6-Month Old Male BXSB Responding Cells

Responder[a]	Stimulator[b]	Incorporation of ^{3}H-TdR (cpm) Unstimulated	Stimulated	Net Incorporation	S.I.[d]
BXSB Male Mice:					
$1x10^6$ 2 mos	$4x10^5$ 2 mos	14,679 (1,859)[c]	26,158 (5,366)	11,479	1.8
$1x10^6$ 2 mos	$8x10^5$ 2 mos	14,679 (1,859)	26,386 (557)	11,707	1.8
$1x10^6$ 2 mos	$4x10^5$ 6 mos	14,679 (1,859)	31,756 (2,418)	17,077	2.2
$1x10^6$ 2 mos	$8x10^5$ 6 mos	14,679 (1,859)	32,936 (6,944)	18,257	2.2
$1x10^6$ 6 mos	$4x10^5$ 2 mos	17,195 (366)	16,968 (355)	-227	-
$1x10^6$ 6 mos	$8x10^5$ 2 mos	17,195 (366)	15,133 (2,896)	-2,062	-
$1x10^6$ 6 mos	$4x10^5$ 6 mos	17,195 (366)	15,925 (1,447)	-1,270	-
$1x10^6$ 6 mos	$8x10^5$ 6 mos	17,195 (366)	13,170 (414)	-4,025	-

[a] Non-adherent T-enriched splenic responding cells

[b] Mitomycin-C treated unfractionated syngeneic spleen cells

[c] ( ) = Standard deviation

[d] S.I. = Stimulation index

TABLE 8 Failure of 2-Month Old Female BXSB Splenic Cells to Stimulate 2-Month Old Male BXSB Responding Cells

Responder[a]	Stimulator[b]	Incorporation of ^{3}H-TdR (cpm)		Net Incorporation	S.I.[d]
		Unstimulated	Stimulated		
BXSB Mice:					
$1x10^6_6$ female	$4x10^5_5$ female	4,210 (1,384)[c]	38,607 (3,759)	34,397	9.1
$1x10^6$ female	$8x10^5$ female	4,210 (1,384)	42,287 (88)	38,077	10.1
$1x10^6_6$ female	$4x10^5_5$ male	4,210 (1,384)	41,002 (25)	36,792	9.7
$1x10^6$ female	$8x10^5$ male	4,210 (1,384)	30,225 (199)	26,015	7.2
$1x10^6_6$ male	$4x10^5_5$ female	11,935 (170)	19,905 (2,486)	7,970	1.7
$1x10^6$ male	$8x10^5$ female	11,935 (170)	18,761 (723)	6,826	1.6
$1x10^6_6$ male	$4x10^5_5$ male	11,935 (170)	20,282 (259)	8,347	1.7
$1x10^6$ male	$8x10^5$ male	11,935 (170)	19,453 (392)	7,518	1.6

[a] Non-adherent T-enriched splenic responding cells

[b] Mitomycin-C treated unfractionated syngeneic spleen cells

[c] ( ) = Standard deviation

[d] S.I. = Stimulation index

TABLE 9 Failure of 2-Month Old MRL/n Splenic Cells to Stimulate 2-Month Old MRL/l Responding Cells

Responder[a]	Stimulator[b]	Incorporation of ^{3}H-TdR (cpm) Unstimulated	Stimulated	Net Incorporation	S.I.[d]
$1x10^6$ MRL/n	$4x10^5$ MRL/n	4,270 (6)[c]	14,647 (2,594)	10,377	3.4
$1x10^6$ MRL/n	$8x10^5$ MRL/n	4,270 (6)	15,076 (362)	10,806	3.5
$1x10^6$ MRL/n	$4x10^5$ MRL/l	4,270 (6)	14,137 (760)	9,867	3.3
$1x10^6$ MRL/n	$8x10^5$ MRL/l	4,270 (6)	17,783 (948)	13,513	4.2
$1x10^6$ MRL/l	$4x10^5$ MRL/l	4,080 (186)	6,009 (760)	1,929	1.5
$1x10^6$ MRL/l	$8x10^5$ MRL/l	4,080 (186)	6,932 (1,352)	2,852	1.7
$1x10^6$ MRL/l	$4x10^5$ MRL/n	4,080 (186)	4,083 (519)	3	-
$1x10^6$ MRL/l	$8x10^5$ MRL/n	4,080 (186)	6,088 (2,401)	2,008	1.5

[a] Non-adherent T-enriched splenic responding cells

[b] Mitomycin-C treated unfractionated syngeneic spleen cells

[c] ( ) = Standard deviation

[d] S.I. = Stimulation index

TABLE 10 Inability of 7-Month Old B/W T-Enriched Cells to Inhibit SMLR by 2-Month Old B/W Mice

Responder[a]	Stimulator[b]	Cells Added[c]	Incorporation of ^{3}H-TdR (cpm)		Net Incorporation	S.I.[e]
			Unstimulated[d]	Stimulated		
2 mos B/W Mice:						
$1x10^6$ B/W	$4x10^5$ B/W	None	1,082 (298)[f]	1,600 (526)	518	1.5
$1x10^6$ B/W	$8x10^5$ B/W	None	1,082 (298)	2,194 (125)	1,112	2.0
$1x10^6$ B/W	$4x10^5$ B/W	$2x10^5$ 2 mos B/W	1,600 (134)	2,030 (320)	430	1.3
$1x10^6$ B/W	$8x10^5$ B/W	$2x10^5$ 2 mos B/W	1,600 (134)	2,864 (1,273)	1,264	1.8
$1x10^6$ B/W	$4x10^5$ B/W	$2x10^5$ 7 mos B/W	1,306 (133)	2,233 (341)	927	1.7
$1x10^6$ B/W	$8x10^5$ B/W	$2x10^5$ 7 mos B/W	1,306 (133)	2,390 (318)	1,084	1.8
$1x10^6$ B/W	$4x10^5$ B/W	$4x10^5$ 2 mos B/W	1,380 (36)	2,857 (252)	1,477	2.1
$1x10^6$ B/W	$8x10^5$ B/W	$4x10^5$ 2 mos B/W	1,380 (36)	3,843 (597)	2,463	2.8
$1x10^6$ B/W	$4x10^5$ B/W	$4x10^5$ 7 mos B/W	1,018 (88)	4,188 (395)	3,170	4.1
$1x10^6$ B/W	$8x10^5$ B/W	$4x10^5$ 7 mos B/W	1,018 (88)	3,814 (112)	2,796	3.7

[a] Non-adherent T-enriched splenic responding cells

[b] Mitomycin-C treated unfractionated syngeneic spleen cells

[c] Non-adherent T-enriched splenic cells

[d] Unstimulated cultures consist of responding cells plus cells added

[e] S.I. = Stimulation index

[f] ( ) = Standard deviation

TABLE 11 Effects of Pretreating 3-Month Old B/W Male Responding Population with Low Dose X-rays on SMLR

Responder[a]	Stimulator[b]	Incorporation of ^{3}H-TdR (cpm) Unstimulated	Incorporation of ^{3}H-TdR (cpm) Stimulated	Net Incorporation	S.I.[d]
$1x10^6$ B/W - 0 Rads	$4x10^5$ B/W	1,136 (159)[c]	2,672 (128)	1,536	2.3
$1x10^6$ B/W - 0 Rads	$8x10^5$ B/W	1,136 (159)	2,123 (452)	987	1.9
$1x10^6$ B/W - 25 Rads	$4x10^5$ B/W	546 (33)	1,677 (70)	1,131	3.1
$1x10^6$ B/W - 25 Rads	$8x10^5$ B/W	546 (33)	1,377 (368)	831	2.5
$1x10^6$ B/W - 50 Rads	$4x10^5$ B/W	320 (37)	935 (55)	615	2.9
$1x10^6$ B/W - 50 Rads	$8x10^5$ B/W	320 (37)	676 (101)	356	2.1
$1x10^6$ B/W - 75 Rads	$4x10^5$ B/W	277 (6)	692 (186)	415	2.5
$1x10^6$ B/W - 75 Rads	$8x10^5$ B/W	277 (6)	572 (138)	295	2.1
$1x10^6$ B/W - 100 Rads	$4x10^5$ B/W	262 (39)	338 (15)	76	1.3
$1x10^6$ B/W - 100 Rads	$8x10^5$ B/W	262 (39)	394 (40)	132	1.5

[a] Non-adherent T-enriched splenic responding cells

[b] Mitomycin-C treated unfractionated syngeneic spleen cells

[c] ( ) = Standard deviation

[d] S.I. = Stimulation index

TABLE 12 Effects of Pretreating 3-Month Old B/W Male Responding Population with Low Dose X-rays on SMLR

Responder[a]	Stimulator[b]	Incorporation of ^{3}H-TdR (cpm) Unstimulated	Stimulated	Net Incorporation	S.I.[d]
$1x10^6$ B/W - 0 Rads	$4x10^5$ B/W	4,830 (1,580)[c]	7,840 (2,323)	3,010	1.6
$1x10^6$ B/W - 0 Rads	$8x10^5$ B/W	4,830 (1,580)	7,111 (723)	2,281	1.5
$1x10^6$ B/W - 100 Rads	$4x10^5$ B/W	765 (21)	1,138 (572)	373	1.5
$1x10^6$ B/W - 100 Rads	$8x10^5$ B/W	765 (21)	1,503 (744)	738	2.0
$1x10^6$ B/W - 200 Rads	$4x10^5$ B/W	748 (77)	222 (30)	-526	-
$1x10^6$ B/W - 200 Rads	$8x10^5$ B/W	748 (77)	473 (54)	-275	-
$1x10^6$ B/W - 300 Rads	$4x10^5$ B/W	443 (32)	201 (22)	-242	-
$1x10^6$ B/W - 300 Rads	$8x10^5$ B/W	443 (32)	152 (7)	-291	-
$1x10^6$ B/W - 400 Rads	$4x10^5$ B/W	411 (40)	116 (12)	-295	-
$1x10^6$ B/W - 400 Rads	$8x10^5$ B/W	411 (40)	153 (31)	-258	-

[a] Non-adherent T-enriched splenic responding cells

[b] Mitomycin-C treated unfractionated syngeneic spleen cells

[c] ( ) = Standard deviation

[d] S.I. = Stimulation index

TABLE 13 Effects of Pretreating 8-Month Old B/W Male Responding Population with Low Dose X-rays on SMLR

Responder[a]	Stimulator[b]	Incorporation of ^{3}H-TdR (cpm) Unstimulated	Stimulated	Net Incorporation	S.I.[d]
$1x10^6$ B/W - 0 Rads	$4x10^5$ B/W	4,072 (16)[c]	5,199 (54)	1,127	1.3
$1x10^6$ B/W - 0 Rads	$8x10^5$ B/W	4,072 (16)	5,317 (444)	1,245	1.3
$1x10^6$ B/W - 100 Rads	$4x10^5$ B/W	1,336 (64)	801 (74)	-535	-
$1x10^6$ B/W - 100 Rads	$8x10^5$ B/W	1,336 (64)	1,003 (133)	-333	-
$1x10^6$ B/W - 200 Rads	$4x10^5$ B/W	1,161 (375)	562 (101)	-599	-
$1x10^6$ B/W - 200 Rads	$8x10^5$ B/W	1,161 (375)	599 (138)	-562	-
$1x10^6$ B/W - 300 Rads	$4x10^5$ B/W	587 (56)	301 (3)	-286	-
$1x10^6$ B/W - 300 Rads	$8x10^5$ B/W	587 (56)	470 (36)	-117	-

[a] Non-adherent T-enriched splenic responding cells

[b] Mitomycin-C treated unfractionated syngeneic spleen cells

[c] ( ) = Standard deviation

[d] S.I. = Stimulation index

TABLE 14 Percentage of IgM^+ Cells and $Thy1.2^+$ Cells in NA Cell Population from Nylon Wool Fractionation

Treatment[a]	% Specific Killing[b]	
	BXSB[c]	CBA[d]
Anti-IgM plus complement	42	30
Anti-Thy1.2 plus complement	24	38

[a] Non-adherent T-enriched splenic cells were treated with the various antisera plus complement

[b] Killing after substracting background killing with complement alone

[c] 4-month old BXSB male mice

[d] 4-month old CBA male mice

TABLE 15 Pretreating 4-Month Old BXSB Male Responding Population with Anti-IgM plus Complement

Responder[a]	Stimulator[b]	Incorporation of ^{3}H-TdR (cpm) Unstimulated	Stimulated	Net Incorporation	S.I.[d]
$1x10^6$ BXSB	$4x10^5$ BXSB	5,835 (74)[c]	11,284 (20)	5,449	1.9
$1x10^6$ BXSB	$8x10^5$ BXSB	5,835 (74)	12,454 (528)	6,619	2.1
$1x10^6$ BXSB treated with C'[e]	$4x10^5$ BXSB	7,162 (303)	8,999 (2,739)	1,837	1.3
	$8x10^5$ BXSB	7,162 (303)	13,089 (1,023)	5,927	1.8
$1x10^6$ BXSB treated with anti-IgM + C'	$4x10^5$ BXSB	4,838 (1,086)	5,164 (152)	326	1.1
	$8x10^5$ BXSB	4,838 (1,086)	10,906 (292)	6,068	2.3

[a] Non-adherent T-enriched splenic responding cells

[b] Mitomycin-C treated unfractionated syngeneic spleen cells

[c] ( ) = Standard deviation

[d] S.I. = Stimulation index

[e] C' = Rabbit complement

TABLE 16 Pretreating 4-Month Old BXSB Male Responding Population with Anti-Thy1.2 plus Complement

Responder[a]	Stimulator[b]	Incorporation of ^{3}H-TdR (cpm) Unstimulated	Stimulated	Net Incorporation	S.I.[d]
$1x10^6$ BXSB	$4x10^5$ BXSB	8,550 (593)[c]	7,584 (518)	-966	-
$1x10^6$ BXSB	$8x10^5$ BXSB	8,550 (593)	5,443 (567)	-3,017	-
$1x10^6$ BXSB treated with C'[e]	$4x10^5$ BXSB	7,094 (315)	7,511 (1,539)	417	-
	$8x10^5$ BXSB	7,094 (315)	5,404 (248)	-1,690	-
$1x10^6$ BXSB treated with anti-Thy + C'	$4x10^5$ BXSB	2,989 (194)	2,714 (163)	-275	-
	$8x10^5$ BXSB	2,989 (194)	2,225 (91)	-764	-

[a] Non-adherent T-enriched splenic responding cells

[b] Mitomycin-C treated unfractionated syngeneic spleen cells

[c] ( ) = Standard deviation

[d] S.I. = Stimulation index

[e] C' = Rabbit complement

TABLE 17 Proliferative Responses Against Alloantigens in 4-Month Old and 8-Month Old B/W Male Mice

Responder[a]	Stimulator[b]	Incorporation of ^{3}H-TdR (cpm)		Net Incorporation	S.I.[c]
		Unstimulated[d]	Stimulated		
$1x10^6$ 4 mos B/W	$4x10^5$ C57BL/6	21,046 (2,277)[e]	66,237 (1,241)	45,191	3.1
$1x10^6$ 4 mos B/W	$8x10^5$ C57BL/6	21,274 (945)	63,184 (6,314)	41,910	3.0
$1x10^6$ 8 mos B/W	$4x10^5$ C57BL/6	11,222 (1,421)	40,537 (5,362)	29,315	3.6
$1x10^6$ 8 mos B/W	$8x10^5$ C57BL/6	10,397 (452)	50,990 (2,095)	40,593	4.9

[a] Non-adherent T-enriched splenic responding cells

[b] Mitomycin-C treated unfractionated spleen cells

[c] S.I. = Stimulation index

[d] Unstimulated cultures for MLR consist of responding cells plus syngeneic unfractionated spleen cells

[e] ( ) = Standard deviation

TABLE 18 Adherent Cells from Nylon Wool Fractionation are Unable to Mediate SMLR

Responder	Stimulator[a]	Incorporation of ^{3}H-TdR (cpm) Unstimulated	Stimulated	Net Incorporation	S.I.[b]
Experiment #22:					
$8x10^5$ CBA NA[c]	$4x10^5$ CBA	2,308 (325)[d]	16,421 (2,396)	14,113	7.1
$8x10^5$ CBA NA	$8x10^5$ CBA	2,308 (325)	27,317 (870)	25,009	11.8
$8x10^5$ CBA AD[e]	$4x10^5$ CBA	13,702 (3,435)	9,398 (1,679)	-4,304	-
$8x10^5$ CBA AD	$8x10^5$ CBA	13,702 (3,435)	12,667 (657)	-1,035	-
Experiment #121:					
$8x10^5$ C57BL/6 NA[c]	$4x10^5$ C57BL/6	4,169 (61)	15,042 (3,191)	10,873	3.6
$8x10^5$ C57BL/6 NA	$8x10^5$ C57BL/6	4,169 (61)	15,686 (648)	11,517	3.8
$8x10^5$ C57BL/6 AD[e]	$4x10^5$ C57BL/6	4,648 (670)	2,530 (85)	-2,118	-
$8x10^5$ C57BL/6 AD	$8x10^5$ C57BL/6	4,648 (670)	2,479 (209)	-2,169	-

[a] Mitomycin-C treated unfractionated syngeneic spleen cells

[b] S.I. = Stimulation index

[c] NA = Non-adherent T-enriched splenic responding cells

[d] ( ) = Standard deviation

[e] AD = Adherent cells from nylon wool fractionation

TABLE 19 Splenic Cells which are not Agglutinated by SBA Mediate SMLR

Responder	Stimulator[a]	Incorporation of ^{3}H-TdR (cpm) Unstimulated	Incorporation of ^{3}H-TdR (cpm) Stimulated	Net Incorporation	S.I.[b]
$8x10^5$ C57BL/6 SB$^-$[c]	$2x10^5$ C57BL/6	1,207 (2)[d]	4,345 (868)	3,138	3.6
$8x10^5$ C57BL/6 SB$^-$	$4x10^5$ C57BL/6	1,207 (2)	4,175 (329)	2,968	3.5
$8x10^5$ C57BL/6 SB$^-$	$8x10^5$ C57BL/6	1,207 (2)	6,233 (1,163)	5,026	5.2
$8x10^5$ C57BL/6 SB$^+$[e]	$2x10^5$ C57BL/6	4,814 (37)	3,175 (702)	-1,639	-
$8x10^5$ C57BL/6 SB$^+$	$4x10^5$ C57BL/6	4,814 (37)	3,062 (192)	-1,752	-
$8x10^5$ C57BL/6 SB$^+$	$8x10^5$ C57BL/6	4,814 (37)	3,023 (403)	-1,791	-

[a] Mitomycin-C treated unfractionated syngeneic spleen cells

[b] S.I. = Stimulation index

[c] SB$^-$ = Splenic cells which are not agglutinated by SBA

[d] ( ) = Standard deviation

[e] SB$^+$ = Splenic cells which are agglutinated by SBA

TABLE 20 Pretreatment of Responding Population with Monoclonal Anti-Thy 1.2 plus Complement Abolishes SMLR

Responder[a]	Stimulator[b]	Incorporation of ^{3}H-TdR (cpm) Unstimulated	Stimulated	Net Incorporation	S.I.[d]
$1x10^6$ C57BL/6	$4x10^5$ C57BL/6	3,833 (1,229)[c]	28,736 (247)	24,903	7.5
$1x10^6$ C57BL/6	$8x10^5$ C57BL/6	3,833 (1,229)	34,808 (369)	30,975	9.1
$1x10^6$ C57BL/6 treated with anti-Thy + C'[e]	$8x10^5$ C57BL/6	2,483 (70)	5,030 (410)	2,547	2.0
$1x10^6$ C57BL/6 treated with C'	$8x10^5$ C57BL/6	7,043 (1,790)	38,177 (1,323)	31,134	5.5

[a] Non-adherent T-enriched splenic responding cells

[b] Mitomycin-C treated unfractionated syngeneic spleen cells

[c] ( ) = Standard deviation

[d] S.I. = Stimulation index

[e] C' = Guinea pig complement

TABLE 21

Pretreatment of Responding Cells with Anti-Lyt 1.2 plus Complement Abolishes SMLR

Responder[a]	Stimulator[b]	Incorporation of ^{3}H-TdR (cpm) Unstimulated	Stimulated	Net Incorporation	S.I.[d]
$1x10^6$ C57BL/6	$4x10^5$ C57BL/6	4,161 (1,749)[c]	21,324 (1,410)	17,163	5.1
$1x10^6$ C57BL/6	$8x10^5$ C57BL/6	4,161 (1,749)	25,624 (2,939)	21,463	6.2
$1x10^6$ C57BL/6 treated with C'[e]	$4x10^5$ C57BL/6	1,912 (530)	7,978 (1,754)	6,066	4.2
	$8x10^5$ C57BL/6	1,912 (530)	16,300 (1,010)	14,388	8.5
$1x10^6$ C57BL/6 treated with anti-Lyt 1.2 + C'	$4x10^5$ C57BL/6	3,208 (48)	4,537 (22)	1,329	1.4
	$8x10^5$ C57BL/6	3,208 (48)	7,521 (1,001)	4,313	2.3
$1x10^6$ C57BL/6 treated with anti-Lyt 2.2 + C'	$4x10^5$ C57BL/6	3,947 (127)	13,736 (885)	9,789	3.5
	$8x10^5$ C57BL/6	3,947 (127)	20,072 (415)	16,125	5.1

[a] Non-adherent T-enriched splenic responding cells

[b] Mitomycin-C treated unfractionated syngeneic spleen cells

[c] ( ) = Standard deviation

[d] S.I. = Stimulation index

[e] C' = Rabbit complement

TABLE 22A Effects of Low Dose X-rays on MLR of C57BL/6 Mice

Responder[a]	Stimulator[b]	Incorporation of ^{3}H-TdR (cpm)		Net Incorporation	S.I.[d]
		Unstimulated[c]	Stimulated		
$1x10^6$ C57BL/6 - 0 Rads	$4x10^5$ DBA	9,114 (588)[e]	77,067 (878)	67,953	8.5
$1x10^6$ C57BL/6 - 50 Rads	$4x10^5$ DBA	1,906 (100)	74,718 (2,336)	72,812	39.0
$1x10^6$ C57BL/6 - 100 Rads	$4x10^5$ DBA	963 (61)	72,982 (7,671)	72,019	76.0
$1x10^6$ C57BL/6 - 200 Rads	$4x10^5$ DBA	427 (38)	32,523 (148)	32,096	75.0

[a] Non-adherent T-enriched splenic responding cells

[b] Mitomycin-C treated unfractionated spleen cells

[c] Unstimulated cultures for MLR consist of responding cells plus syngeneic unfractionated spleen cells

[d] S.I. = Stimulation index

[e] ( ) = Standard deviation

TABLE 22B Effects of Low Dose X-rays on SMLR of C57BL/6 Mice

Responder[a]	Stimulator[b]	Incorporation of ^{3}H-TdR (cpm) Unstimulated	Stimulated	Net Incorporation	S.I.[d]
$1x10^6$ C57BL/6 - 0 Rads	$4x10^5$ C57BL/6	1,553 (163)[c]	17,175 (5,982)	15,622	11.0
$1x10^6$ C57BL/6 - 0 Rads	$8x10^5$ C57BL/6	1,553 (163)	14,741 (1,212)	13,188	9.5
$1x10^6$ C57BL/6 - 50 Rads	$4x10^5$ C57BL/6	530 (5)	3,640 (1,564)	3,110	6.9
$1x10^6$ C57BL/6 - 50 Rads	$8x10^5$ C57BL/6	530 (5)	6,171 (121)	5,641	11.6
$1x10^6$ C57BL/6 - 100 Rads	$4x10^5$ C57BL/6	248 (13)	966 (11)	718	3.9
$1x10^6$ C57BL/6 - 100 Rads	$8x10^5$ C57BL/6	248 (13)	1,717 (81)	1,469	7.0
$1x10^6$ C57BL/6 - 200 Rads	$4x10^5$ C57BL/6	243 (11)	470 (272)	227	1.9
$1x10^6$ C57BL/6 - 200 Rads	$8x10^5$ C57BL/6	243 (11)	534 (31)	291	2.2

[a] Non-adherent T-enriched splenic responding cells

[b] Mitomycin-C treated unfractionated syngeneic spleen cells

[c] ( ) = Standard deviation

[d] S.I. = Stimulation index

TABLE 23A Effects of Low Dose X-rays on MLR of 4-Month Old B/W Male Mice

Responder[a]	Stimulator[b]	Incorporation of ^{3}H-TdR (cpm)		Net Incorporation	S.I.[c]
		Unstimulated[d]	Stimulated		
$1x10^6$ B/W - 0 Rads	$4x10^5$ CBA	8,398 (139)[e]	31,974 (115)	23,576	3.8
$1x10^6$ B/W - 50 Rads	$4x10^5$ CBA	5,302 (231)	24,481 (231)	19,179	4.6
$1x10^6$ B/W - 100 Rads	$4x10^5$ CBA	3,557 (134)	19,391 (2,220)	15,834	5.5
$1x10^6$ B/W - 200 Rads	$4x10^5$ CBA	1,472 (98)	12,226 (199)	10,754	8.3

[a] Non-adherent T-enriched splenic responding cells

[b] Mitomycin-C treated unfractionated spleen cells

[c] S.I. = Stimulation index

[d] Unstimulated cultures for MLR consist of responding cells plus syngeneic unfractionated spleen cells

[e] ( ) = Standard deviation

TABLE 23B Effects of Low Dose X-rays on SMLR of 4-Month Old B/W Male Mice

Responder[a]	Stimulator[b]	Incorporation of ^{3}H-TdR (cpm) Unstimulated	Stimulated	Net Incorporation	S.I.[c]
$1x10^6$ B/W - 0 Rads	$4x10^5$ B/W	4,430 (515)[d]	11,096 (1,238)	6,666	2.5
$1x10^6$ B/W - 0 Rads	$8x10^5$ B/W	4,430 (515)	10,100 (1,065)	5,670	2.3
$1x10^6$ B/W - 50 Rads	$4x10^5$ B/W	3,351 (162)	5,013 (1,067)	1,662	1.5
$1x10^6$ B/W - 50 Rads	$8x10^5$ B/W	3,351 (162)	5,436 (149)	2,085	1.6
$1x10^6$ B/W - 100 Rads	$4x10^5$ B/W	2,054 (23)	3,045 (183)	991	1.5
$1x10^6$ B/W - 100 Rads	$8x10^5$ B/W	2,054 (23)	4,894 (69)	2,840	2.4
$1x10^6$ B/W - 200 Rads	$4x10^5$ B/W	1,248 (76)	1,023 (164)	225	-
$1x10^6$ B/W - 200 Rads	$8x10^5$ B/W	1,248 (76)	1,276 (349)	28	-

[a] Non-adherent T-enriched splenic responding cells

[b] Mitomycin-C treated unfractionated syngeneic spleen cells

[c] S.I. = Stimulation index

[d] ( ) = Standard deviation

TABLE 24A

Effects of in vivo Pretreatment with High-Dose Cyclophosphamide on MLR of C57BL/6 Mice

Responder[a]	Stimulator[b]	Incorporation of ^{3}H-TdR (cpm)		Net Incorporation	S.I.[d]
		Unstimulated[c]	Stimulated		
$1x10^6$ C57BL/6 (PBS)	$4x10^5$ DBA	9,508 (931)[e]	155,204 (11,213)	145,696	16.3
$1x10^6$ C57BL/6 (cyclophosphamide)	$4x10^5$ DBA	1,527 (201)	110,743 (607)	109,216	72.5

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[a] Non-adherent T-enriched splenic responding cells

PBS = pretreated with PBS
cyclophosphamide = pretreated with cyclophosphamide

[b] Mitomycin-C treated unfractionated spleen cells

[c] Unstimulated cultures for MLR consist of responding cells plus syngeneic unfractionated spleen cells

[d] S.I. = Stimulation index

[e] ( ) = Standard deviation

TABLE 24B

Effects of in vivo Pretreatment with High-Dose Cyclophosphamide on SMLR of C57BL/6 Mice

Responder	Stimulator[b]	Incorporation of ^{3}H-TdR (cpm) Unstimulated	Incorporation of ^{3}H-TdR (cpm) Stimulated	Net Incorporation	S.I.[d]
C57BL/6 Mice:					
$1x10^6$ NA[a] (PBS)	$4x10^5$ "PBS"	3,764 (22)[c]	18,038 (158)	14,274	4.8
	$8x10^5$ "PBS"	3,764 (22)	16,419 (156)	12,655	4.4
$1x10^6$ NA (PBS)	$4x10^5$ "cyclophos"	3,764 (22)	11,402 (991)	7,638	3.0
	$8x10^5$ "cyclophos"	3,764 (22)	10,079 (2,298)	6,315	2.7
$1x10^6$ NA (cyclophos)	$4x10^5$ "PBS"	954 (12)	1,678 (2)	724	1.8
	$8x10^5$ "PBS"	954 (12)	3,203 (405)	2,249	3.4
$1x10^6$ NA (cyclophos)	$4x10^5$ "cyclophos"	954 (12)	1,119 (493)	165	1.2
	$8x10^5$ "cyclophos"	954 (12)	1,899 (265)	945	2.0

[a] NA = Non-adherent T-enriched splenic responding cells

[b] Mitomycin-C treated unfractionated syngeneic spleen cells

[c] ( ) = Standard deviation

[d] S.I. = Stimulation index

PBS = pretreated with PBS
cyclophos = pretreated with cyclophosphamide

TABLE 25A

Effects of in vivo Pretreatment with Low-Dose Cyclophosphamide on MLR of C57BL/6 Mice

Responder[a]	Stimulator[b]	Incorporation of ^{3}H-TdR (cpm)		Net Incorporation	S.I.[d]
		Unstimulated[c]	Stimulated		
$1x10^6$ C57BL/6 (PBS)	$4x10^5$ DBA	5,924 (191)[e]	89,285 (32)	83,361	15.1
$1x10^6$ C57BL/6 (cyclophosphamide)	$4x10^5$ DBA	11,288 (249)	93,981 (115)	82,693	8.3

PBS = pretreated with PBS
cyclophosphamide = pretreated with cyclophosphamide

[a] Non-adherent T-enriched splenic responding cells

[b] Mitomycin-C treated unfractionated spleen cells

[c] Unstimulated cultures for MLR consist of responding cells plus syngeneic unfractionated spleen cells

[d] S.I. = Stimulation index

[e] ( ) = Standard deviation

TABLE 25B

Effects of in vivo Pretreatment with Low-Dose Cyclophosphamide on SMLR of C57BL/6 Mice

Responder	Stimulator[b]	Incorporation of ^{3}H-TdR (cpm) Unstimulated	Stimulated	Net Incorporation	S.I[d]
C57BL/6 Mice:					
$1x10^6$ NA[a] (PBS)	$4x10^5$ "PBS"	2,648 (326)[c]	12,654 (748)	10,006	4.8
	$8x10^5$ "PBS"	2,648 (326)	15,412 (1,334)	12,764	5.9
$1x10^6$ NA (PBS)	$4x10^5$ "cyclophos"	2,648 (326)	12,288 (3,400)	9,640	4.6
	$8x10^5$ "cyclophos"	2,648 (326)	12,822 (1,630)	10,174	4.8
$1x10^6$ NA (cyclophos)	$4x10^5$ "PBS"	3,176 (253)	14,765 (129)	11,589	4.6
	$8x10^5$ "PBS"	3,176 (253)	17,271 (250)	14,095	5.4
$1x10^6$ NA (cyclophos)	$4x10^5$ "cyclophos"	3,176 (253)	13,573 (283)	10,397	4.3

[a] NA = Non-adherent T-enriched splenic responding cells

[b] Mitomycin-C treated unfractionated syngeneic spleen cells

[c] ( ) = Standard deviation

[d] S.I. = Stimulation index

PBS = pretreated with PBS
cyclophos = pretreated with cyclophosphamide

TABLE 26 In vitro Effects of Hydrocortisone on SMLR of C57BL/6 Mice

Responder[a]	Stimulator[b]	Incorporation of ^{3}H-TdR (cpm) Unstimulated	Stimulated	Net Incorporation	S.I.[d]
$8x10^5$ C57BL/6	$4x10^5$ C57BL/6	3,262 (955)[c]	14,932 (1,712)	11,670	4.6
$8x10^5$ C57BL/6	$8x10^5$ C57BL/6	3,262 (955)	16,571 (3,234)	13,309	5.1
$8x10^5$ C57BL/6-$8x10^{-6}$M HC[e]	$4x10^5$ C57BL/6	148 (1)	199 (4)	51	-
$8x10^5$ C57BL/6-$8x10^{-6}$M HC	$8x10^5$ C57BL/6	148 (1)	133 (10)	-15	-
$8x10^5$ C57BL/6-$8x10^{-7}$M HC	$4x10^5$ C57BL/6	283 (18)	426 (66)	143	1.5
$8x10^5$ C57BL/6-$8x10^{-7}$M HC	$8x10^5$ C57BL/6	283 (18)	349 (49)	66	1.2
$8x10^5$ C57BL/6-$8x10^{-8}$M HC	$4x10^5$ C57BL/6	1,874 (215)	1,358 (240)	-516	-
$8x10^5$ C57BL/6-$8x10^{-8}$M HC	$8x10^5$ C57BL/6	1,874 (215)	2,365 (290)	491	1.3
$8x10^5$ C57BL/6-$8x10^{-9}$M HC	$4x10^5$ C57BL/6	5,031 (518)	10,754 (1,469)	5,723	2.1
$8x10^5$ C57BL/6-$8x10^{-9}$M HC	$8x10^5$ C57BL/6	5,031 (518)	16,065 (378)	11,034	3.2

[a] Non-adherent T-enriched splenic responding cells

[b] Mitomycin-C treated unfractionated syngeneic spleen cells

[c] ( ) = Standard deviation

[d] S.I. = Stimulation index

[e] HC = hydrocortisone

TABLE 27 In vitro Effects of Hydrocortisone on MLR of C57BL/6 Mice

Responder[a]	Stimulator[b]	Incorporation of ^{3}H-TdR (cpm)		Net Incorporation	S.I.[d]
		Unstimulated[c]	Stimulated		
$8x10^5$ C57BL/6	$4x10^5$ DBA	4,556 (1,252)[e]	98,008 (1,834)	93,452	21.5
$8x10^5$ C57BL/6-$8x10^{-6}$M HC[f]	$4x10^5$ DBA	82 (11)	17,087 (204)	17,005	208.0
$8x10^5$ C57BL/6-$8x10^{-7}$M HC	$4x10^5$ DBA	156 (11)	35,740 (1,766)	35,584	228.0
$8x10^5$ C57BL/6-$8x10^{-8}$M HC	$4x10^5$ DBA	833 (33)	81,089 (7,133)	80,256	97.0
$8x10^5$ C57BL/6-$8x10^{-9}$M HC	$4x10^5$ DBA	3,955 (246)	105,670 (3,945)	101,715	26.7

[a] Non-adherent T-enriched splenic responding cells

[b] Mitomycin-C treated unfractionated spleen cells

[c] Unstimulated cultures for MLR consist of responding cells plus syngeneic unfractionated spleen cells

[d] S.I. = Stimulation index

[e] ( ) = Standard deviation

[f] HC = Hydrocortisone

TABLE 28A Both PNA^+ and PNA^- T Cells can Mediate MLR in C57BL/6 Mice

Responder[a]	Stimulator[b]	Incorporation of ^{3}H-TdR (cpm)		Net Incorporation	S.I.[c]
		Unstimulated[d]	Stimulated		
$1x10^6$ C57BL/6	$4x10^5$ DBA	6,884 (2,120)[e]	86,264 (3,155)	79,380	12.5
$1x10^6$ C57BL/6 PNA^-	$4x10^5$ DBA	3,308 (105)	128,786 (821)	125,478	39.0
$1x10^6$ C57BL/6 PNA^+	$4x10^5$ DBA	10,244 (418)	115,208 (375)	104,964	11.2

[a] Non-adherent T-enriched splenic responding cells
PNA^- = Non-adherent T cells which are not agglutinated by PNA
PNA^+ = Non-adherent T cells which are agglutinated by PNA

[b] Mitomycin-C treated unfractionated spleen cells

[c] S.I. = Stimulation index

[d] Unstimulated cultures for MLR consist of responding cells plus syngeneic unfractionated spleen cells

[e] ( ) = Standard deviation

TABLE 28B Both PNA^+ and PNA^- T Cells can Mediate SMLR in C57BL/6 Mice

Responder[a]	Stimulator[b]	Incorporation of ^{3}H-TdR (cpm)		Net Incorporation	S.I.[d]
		Unstimulated	Stimulated		
$1x10^6$ C57BL/6	$4x10^5$ C57BL/6	1,745 (275)[c]	16,713 (1,710)	14,968	9.6
$1x10^6$ C57BL/6	$8x10^5$ C57BL/6	1,745 (275)	30,075 (748)	28,330	17.2
$1x10^6$ C57BL/6 PNA^-	$4x10^5$ C57BL/6	1,054 (232)	8,559 (2,344)	7,505	8.1
$1x10^6$ C57BL/6 PNA^-	$8x10^5$ C57BL/6	1,054 (232)	22,778 (1,213)	21,724	21.6
$1x10^6$ C57BL/6 PNA^+	$4x10^5$ C57BL/6	2,981 (274)	32,652 (6,101)	29,671	11.0
$1x10^6$ C57BL/6 PNA^+	$8x10^5$ C57BL/6	2,981 (274)	26,404 (3,342)	23,423	8.9

[a] Non-adherent T-enriched splenic responding cells

PNA^- = Non-adherent T cells which are not agglutinated by PNA

PNA^+ = Non-adherent T cells which are agglutinated by PNA

[b] Mitomycin-C treated unfractionated syngeneic spleen cells

[c] ( ) = Standard deviation

[d] S.I. = Stimulation index

TABLE 29A Both PNA^+ and PNA^- T Cells can Mediate MLR in 9-Month Old B/W Male Mice

Responder[a]	Stimulator[b]	Incorporation of ^{3}H-TdR (cpm)		Net Incorporation	S.I.[c]
		Unstimulated[d]	Stimulated		
$1x10^6$ B/W	$4x10^5$ C57BL/6	5,685 (93)[e]	18,412 (246)	12,727	3.2
$1x10^6$ B/W PNA^-	$4x10^5$ C57BL/6	2,581 (25)	17,686 (600)	15,105	6.9
$1x10^6$ B/W PNA^+	$4x10^5$ C57BL/6	4,288 (1,032)	20,284 (38)	15,996	4.7

[a] Non-adherent T-enriched splenic responding cells

PNA^- = Non-adherent T cells which are not agglutinated by PNA
PNA^+ = Non-adherent T cells which are agglutinated by PNA

[b] Mitomycin-C treated unfractionated spleen cells

[c] S.I. = Stimulation index

[d] Unstimulated cultures for MLR consist of responding cells plus syngeneic unfractionated spleen cells

[e] ( ) = Standard deviation

TABLE 29B Both PNA^+ and PNA^- T Cells can Mediate SMLR in 9-Month Old B/W Male Mice

Responder[a]	Stimulator[b]	Incorporation of ^{3}H-TdR (cpm) Unstimulated	Stimulated	Net Incorporation	S.I.[d]
$1x10^6$ B/W	$4x10^5$ B/W	6,928 (2)[c]	6,476 (468)	-452	-
$1x10^6$ B/W	$8x10^5$ B/W	6,928 (2)	6,207 (594)	-721	-
$1x10^6$ B/W PNA^-	$4x10^5$ B/W	2,790 (6)	3,647 (62)	857	1.3
$1x10^6$ B/W PNA^-	$8x10^5$ B/W	2,790 (6)	4,766 (219)	1,976	1.7
$1x10^6$ B/W PNA^+	$4x10^5$ B/W	6,065 (2,732)	7,386 (332)	1,321	1.2
$1x10^6$ B/W PNA^+	$8x10^5$ B/W	6,065 (2,732)	6,522 (1,604)	457	1.1

[a] Non-adherent T-enriched splenic responding cells

PNA^- = Non-adherent T cells which are not agglutinated by PNA

PNA^+ = Non-adherent T cells which are agglutinated by PNA

[b] Mitomycin-C treated unfractionated syngeneic spleen cells

[c] ( ) = Standard deviation

[d] S.I. = Stimulation index

TABLE 30 SMLR Against Spleen Cells, Bone Marrow Cells, Lymph Node Cells, or Thymocytes

Responder	Stimulator[b]	Incorporation of ^{3}H-TdR (cpm) Unstimulated	Incorporation of ^{3}H-TdR (cpm) Stimulated	Net Incorporation	S.I.[d]
C57BL/6 Mice:					
$1x10^6$ NA[a]	$4x10^5$ SPC	3,962 (915)[c]	11,941 (2,243)	7,979	3.0
$1x10^6$ NA	$8x10^5$ SPC	3,962 (915)	15,440 (509)	11,478	4.0
$1x10^6$ NA	$4x10^5$ BMC	3,962 (915)	5,062 (748)	1,100	1.3
$1x10^6$ NA	$8x10^5$ BMC	3,962 (915)	2,985 (903)	-977	-
$1x10^6$ NA	$4x10^5$ LNC	3,962 (915)	4,911 (974)	949	1.2
$1x10^6$ NA	$8x10^5$ LNC	3,962 (915)	7,953 (1,702)	3,991	2.0
$1x10^6$ NA	$4x10^5$ THY	3,962 (915)	2,787 (152)	-1,175	-
$1x10^6$ NA	$8x10^5$ THY	3,962 (915)	2,786 (37)	-1,176	-

[a] NA = Non-adherent T-enriched splenic responding cells

[b] Mitomycin-C treated syngeneic cells: SPC = Spleen cells BMC = Bone marrow cells LNC = Lymph node cells THY = Thymocytes

[c] ( ) = Standard deviation

[d] S.I. = Stimulation index

TABLE 31 SMLR Against Adherent Splenic Cells from Nylon Wool Fractionation

Responder	Stimulator[b]	Incorporation of ^{3}H-TdR (cpm) Unstimulated	Stimulated	Net Incorporation	S.I.[d]
CBA Mice:					
$8x10^5_5$ NA[a]	$4x10^5_5$ unfrac	1,248 (37)[c]	1,924 (305)	676	1.5
$8x10^5$ NA	$8x10^5$ unfrac	1,248 (37)	6,543 (309)	5,295	5.2
$8x10^5_5$ NA	$4x10^5_5$ NA_m	1,248 (37)	1,423 (347)	175	1.1
$8x10^5$ NA	$8x10^5$ NA_m	1,248 (37)	2,740 (511)	1,492	2.2
$8x10^5_5$ NA	$4x10^5_5$ AD	1,248 (37)	9,882 (447)	8,634	8.0
$8x10^5$ NA	$8x10^5$ AD	1,248 (37)	8,983 (908)	7,735	7.2

[a] NA = Non-adherent T-enriched splenic responding cells

[b] Mitomycin-C treated syngeneic spleen cells: unfrac = Unfractionated spleen cells
NA_m = Non-adherent fraction of spleen cells
AD = Adherent fraction of spleen cells

[c] ( ) = Standard deviation

[d] S.I. = Stimulation index

TABLE 32 The Stimulating Cell of SMLR is Agglutinated by SBA

Responder	Stimulator[b]	Incorporation of ^{3}H-TdR (cpm) Unstimulated	Incorporation of ^{3}H-TdR (cpm) Stimulated	Net Incorporation	S.I.[d]
C57BL/6 Mice:					
$8x10^5$ SB^-[a]	$2x10^5$ unfrac	1,207 (21)[c]	4,345 (868)	3,138	3.6
$8x10^5$ SB^-	$4x10^5$ unfrac	1,207 (21)	4,175 (329)	2,968	3.5
$8x10^5$ SB^-	$8x10^5$ unfrac	1,207 (21)	6,233 (1,163)	5,026	5.2
$8x10^5$ SB^-	$2x10^5$ SB^+ cells	1,207 (21)	4,501 (1,264)	3,294	3.7
$8x10^5$ SB^-	$4x10^5$ SB^+ cells	1,207 (21)	6,583 (1,038)	5,376	5.5
$8x10^5$ SB^-	$8x10^5$ SB^+ cells	1,207 (21)	11,831 (114)	10,624	9.8

[a] SB^- = Splenic cells which are not agglutinated by SBA (T-enriched fraction)

[b] Mitomycin-C treated spleen cells: Unfrac = Unfractionated spleen cells
SB^+ = Spleen cells which are agglutinated by SBA

[c] ( ) = Standard deviation

[d] S.I. = Stimulation index

TABLE 33 SMLR Against PNA^+ and PNA^- Spleen Cells

Responder	Stimulator[b]	Incorporation of ^{3}H-TdR (cpm) Unstimulated	Incorporation of ^{3}H-TdR (cpm) Stimulated	Net Incorporation	S.I.[d]
C57BL/6 Mice:					
$1x10^6$ NA[a]	$4x10^5$ unfrac	5,449 (183)[c]	13,189 (1,993)	7,740	2.4
$1x10^6$ NA	$8x10^5$ unfrac	5,449 (183)	13,237 (3,310)	7,788	2.4
$1x10^6$ NA	$4x10^5$ PNA^+ cells	5,449 (183)	16,804 (3,013)	11,355	3.1
$1x10^6$ NA	$8x10^5$ PNA^+ cells	5,449 (183)	28,496 (8,833)	23,047	5.2
$1x10^6$ NA	$4x10^5$ PNA^- cells	5,449 (183)	8,917 (969)	3,468	1.6
$1x10^6$ NA	$8x10^5$ PNA^- cells	5,449 (183)	12,607 (3,116)	7,158	2.3

[a] NA = Non-adherent T-enriched splenic responding cells

[b] Mitomycin-C treated syngeneic spleen cells: unfrac = Unfractionated spleen cells
PNA^+ = Spleen cells which are agglutinated by PNA
PNA^- = Spleen cells which are not agglutinated by PNA

[c] ( ) = Standard deviation

[d] S.I. = Stimulation index

TABLE 34 Spleen Cells Depleted of Adherent Cells are Able to Stimulate SMLR

Responder	Stimulator[b]	Incorporation of ^{3}H-TdR (cpm) Unstimulated	Incorporation of ^{3}H-TdR (cpm) Stimulated	Net Incorporation	S.I.[d]
C57BL/6 Mice:					
$1x10^6$ NA[a]	$4x10^5$ unfrac	995 (264)[c]	8,565 (1,350)	7,570	8.6
$1x10^6$ NA	$8x10^5$ unfrac	995 (264)	18,097 (3,690)	17,102	18.2
$1x10^6$ NA	$4x10^5$ frac	995 (264)	13,385 (1,318)	12,390	13.4
$1x10^6$ NA	$8x10^5$ frac	995 (264)	23,007 (1,530)	22,012	23.1

[a] NA = Non-adherent T-enriched splenic responding cells

[b] Mitomycin-C treated syngeneic spleen cells: unfrac = Unfractionated spleen cells
frac = Spleen fraction depleted of cells adhering to plastics

[c] ( ) = Standard deviation

[d] S.I. = Stimulation index

TABLE 35 SMLR and MLR Against Non-adherent Cells from Spleen Cultures

SMLR

Responder[a]	Stimulator[b]	Incorporation of ^{3}H-TdR (cpm)		Net Incorporation	S.I.[d]
		Unstimulated	Stimulated		
$1x10^6$ C57BL/6	$4x10^5$ C57BL/6 uncult	3,408 (240)[c]	9,002 (354)	5,594	2.6
$1x10^6$ C57BL/6	$8x10^5$ C57BL/6 uncult	3,408 (240)	9,671 (112)	6,263	2.8
$1x10^6$ C57BL/6	$4x10^5$ C57BL/6 cult	3,408 (240)	16,409 (27)	13,001	4.8
$1x10^6$ C57BL/6	$8x10^5$ C57BL/6 cult	3,408 (240)	17,571 (267)	14,163	5.2

MLR

Responder[a]	Stimulator[b]	Incorporation of ^{3}H-TdR (cpm)		Net Incorporation	S.I.[d]
		Unstimulated[e]	Stimulated		
$1x10^6$ C57BL/6	$4x10^5$ DBA uncult	9,900 (327)[c]	140,868 (2,525)	130,968	14.2
$1x10^6$ C57BL/6	$8x10^5$ DBA uncult	19,625 (658)	154,945 (2,885)	135,320	7.9
$1x10^6$ C57BL/6	$4x10^5$ DBA cult	13,661 (1,006)	120,925 (1,240)	107,264	8.9
$1x10^6$ C57BL/6	$8x10^5$ DBA cult	19,667 (7,418)	78,827 (6,176)	59,160	4.0

[a] Non-adherent T-enriched splenic responding cells

[b] Mitomycin-C treated syngeneic spleen cells: uncult = Uncultured and unfractionated spleen cells
cult = Non-adherent fraction from cultured spleen cells

[c] ( ) = Standard deviation

[d] S.I. = Stimulation index

[e] Cocultures of responding cells plus cultured syngeneic splenic stimulating cells were employed as unstimulated cultures for MLR against cultured allogeneic cells

DISCUSSION

I. DECREASED SMLR in AUTOIMMUNE MICE

In this thesis, the SMLR was employed to search for a common immunological alteration shared by autoimmune-susceptible mice. The results reported here suggest that a decreased SMLR is a common feature in several autoimmune mouse strains, including the B/W and the newly developed BXSB and MRL/l. The SMLR represents a proliferative response by splenic T-enriched cells to unfractionated syngeneic spleen cells that had been treated with mitomycin-C. Experiments utilizing selective killing with anti-Thyl.2 plus complement and SBA separation techniques have confirmed previous reports that the cell mediating SMLR is a T cell (Smith and Pasternak, 1978). The stimulating cell involved in SMLR has not been well characterized, but non-T splenic cells are capable of stimulating SMLR.

A decrease in SMLR is observed in autoimmune-susceptible strains before the appearance of clinical symptoms. For example, at 2 months of age, when autoimmune disease is not apparent, the SMLR is already lowered in B/W females. When they begin to show signs of disease at 6 months of age, the SMLR declines to an undetectable level. Likewise, in B/W males which begin to develop autoimmunity at about 9 months of age (Papoian et al, 1977; Roubinian et al, 1977), the SMLR is normal at 2 months but reduced at 7 months. In the BXSB males, the SMLR declines markedly at 2 months before the appearance of autoimmune disease. The SMLR of MRL/l males is barely detectable at 2 months of age. In MRL/n mice, which develop autoimmune disease later, the SMLR is variable at

2 months of age, but is greater than in MRL/l mice. Hence, an impairment in SMLR occurs in autoimmune mice prior to the onset of clinical disease.

The diminished SMLR present in autoimmune mice may relate to disease severity, since it is most pronounced in autoimmune mice which express a severe form of the disease. An accelerated expression of the lupus-like disorder occurs in B/W females and BXSB males reflecting the early onset of disease in B/W females and in BXSB males. In the B/W mice, the disease appears in females at 6 months of age, whereas males develop the disease much later in life. A 50% mortality occurs at 8-1/2 months for females and 15 months for males. Unlike the B/W mice, BXSB males begin to show signs of disease very early. The disease occurs later in BXSB females. The 50% mortality is 5 months for BXSB males compared to 15 months for BXSB females. Similarly, in the B/W strain, the decreased SMLR is more pronounced in females; by contrast, in the BXSB strain, the SMLR is much lower in males. Female and male C57BL/6 mice give fairly equal responses in SMLR.

The difference in SMLR between female and male BXSB mice is greater than between female and male B/W mice. Perhaps this is because BXSB females have fairly strong SMLR similar to that of C57BL/6 mice. Furthermore, the lupus-like disease of BXSB males is more acute than that of B/W females. In most experiments, the incorporation of H^3-TdR by unstimulated cultures of BXSB mice was high, particularly for BXSB males. This high background proliferation may contribute to the lowered SMLR observed in BXSB males. The reason for such a high background proliferation in unstimulated cultures is unknown, but the type of nutrient serum used in the cultures appears to influence the magnitude of back-

ground proliferation obtained. In general, high incorporation of H^3-TdR in unstimulated cultures is observed, with autoimmune as well as non-autoimmune mouse strains when employing fetal calf serum. This background proliferation, however, is greatly reduced when HS is utilized, especially in non-autoimmune mouse cultures.

The spleens of all autoimmune mouse strains appear to manifest alterations in their T and B cell composition with increasing age and advanced disease (Theofilopoulous et al, 1979). Such alterations in the proportion of T and B cells within the spleen may contribute to the high incorporation of H^3-TdR in unstimulated cultures of these autoimmune mice. For example, the BXSB males and not the females possess increased frequencies and also absolute numbers of Ig^+ cells associated with advanced disease. BXSB mice also have a lymphoproliferative disorder predominantly characterized by the presence of lymphocytes of B cell origin (Murphy and Roths, 1978). In addition, a reduced number of T cells occurs in BXSB mice with advanced age and disease (Theofilopoulous et al, 1979). Since BXSB spleens have increased numbers of B cells and decreased numbers of T cells compared to spleens of normal mice, fractionation of BXSB spleen cells on nylon wool may give a NA cell preparation containing altered proportions of T and B cells. An excessive number of B cells in BXSB spleens can pass through nylon wool; therefore, such BXSB NA cell fractions may possess higher numbers of contaminating B cells or even macrophages or null cells than NA cell fractions from normal spleen cells. Results presented in this thesis suggest that this view is possible. Enumerations on the percentage of IgM^+ cells and $Thy1.2^+$ cells within the NA cell population from nylon wool fractionation indicate that the NA cell population of BXSB males contain fewer T cells

and higher numbers of IgM^+ cells than that of the normal strain, CBA. Immunofluorescent studies also show elevated numbers of Ig^+ cells in BXSB NA-cell fractions compared to that of CBA (not reported here). An increase in B cells within the NA-cell fraction may be responsible for the high background proliferation observed in unstimulated cultures of BXSB mice. Moreover, these contaminating B cells are able to stimulate syngeneic T cells in the NA cell population, and thereby, result in the occurrence of an ongoing SMLR in cultures containing only BXSB NA-responding cells. Hence, the increased background proliferation in cultures of NA-responding cells to which no mitomycin-C treated syngeneic spleen cells were added actually represents a SMLR.

It was predicted that if these contaminating B cells were responsible for the high background proliferation, then the removal of these cells would lead to a reduction in the background proliferation and possibly an improvement in SMLR by BXSB mice. Selective elimination of the high numbers of contaminating B cells in the NA-cell fraction of BXSB mice was achieved by pretreating the NA-responding cells with anti-IgM plus complement. It was observed that anti-IgM plus complement pretreatment of BXSB responding cells did not alter the background proliferation and furthermore, did not enhance the SMLR of BXSB mice. These findings indicate that the IgM^+ cells do not contribute to the high background proliferation manifested by BXSB mice; however, it is possible that B cells bearing other surface immunoglobulins such as IgG may be involved. Further studies employing anti-IgG plus complement pretreatment of the responding cells are required to determine whether this is the case.

The majority of the high background proliferation observed in unstimulated cultures of BXSB responding cells, however, appears to be mediated by T cells. This view is suggested by the observation that when BXSB responding cell population was pretreated with anti-Thy1.2 plus complement, the background proliferation was greatly lowered. However, a moderate reduction in the background proliferation was observed in unstimulated cultures containing C57BL/6 responding cells which were treated with anti-Thy1.2 plus complement. Furthermore, this high background proliferation which is sensitive to the effect of anti-Thy1 and complement may reflect the activities of preactivated T cells present in BXSB mice. Experiments in which preactivated T cells are separated from nonactivated T cells hopefully can resolve whether the presence of preactivated T cells in BXSB NA-cell populations contributes to the high background proliferation. If such experiments indicate that the high background proliferation of BXSB mice is due to the presence of preactivated T cells, it would be interesting to determine whether these T cells can serve as stimulators of SMLR. It is possible that such activated T cells may express altered antigenic determinants on their membranes which could stimulate other nonactivated T cell and thereby bring about an ongoing SMLR in BXSB cultures containing only responding cells.

The alterations in the T and B cell compositions of the spleen are not the same in all autoimmune strains (Theofilopoulous et al, 1979). Unlike the BXSB mice, NZB and B/W mice have reduced frequencies and numbers of Ig^+ cells in the spleen and lymph node with advanced age and disease. The numbers of T cells are also decreased in lymphoid organs of NZB and B/W mice. These findings of decreased T and B cells

in the spleen of NZ mice tend not to support the view that the NA-cell fraction of NZ mice may contain increasing numbers of contaminating B cells which can cause the high background proliferation. The presence of reduced numbers of T and B cells, however, suggest that the spleen of the NZ mice may possess elevated numbers of null cells. In fact, the activity of natural killer cells, which lack characteristics of mature T or B cells and hence defined as null cells, is increased in B/W mice (Seaman et al, 1978). An increase in the number of null cells can lead to high levels of natural killing in B/W mice. Along similar lines, Stobo et al (1972) also reported an increased frequency of splenic lymphocytes lacking immunoglobulin or theta determinants in NZ mice. Therefore, it is conceivable that the high background proliferation observed in NZ mice may be due to contaminating null cells within the NA-cell population from nylon wool fractionation. In order to substantiate the presence of contaminating null cells, it would be necessary to determine whether NA-cell populations from autoimmune mice can mediate natural killing functions.

The MRL/l mice, on the other hand, have a lymphoproliferative disorder involving mainly T cells. With advanced disease, an increase in T cell frequencies and massive increases in the absolute numbers of T cells occur in the MRL/l mice. In this case, the presence of pre-activated T cells in the NA-cell preparation may account for the high background proliferation of MRL/l mice.

At the present time, the causes for the high background proliferation in unstimulated cultures of autoimmune mice cannot be fully defined. As discussed above, a variety of factors can bring about the high background proliferation. Further investigations on the causes for this

high background proliferation would be worthwhile, since the high background proliferation may contribute to the lowered SMLR observed in autoimmune mice. In some experiments, however, even though the background proliferations of 6-month old BXSB female and 2-month old MRL/n mice are fairly high, a strong SMLR is still observed in cultures of these mice.

II. NATURE of the DEFECT in AUTOIMMUNE MICE

The cellular basis for the decreased SMLR of autoimmune mice resides within the responding T cell population. Various reciprocal cell mixing experiments were performed to determine the cell responsible for the diminished SMLR present in autoimmune mice. In B/W mice, young and old B/W unfractionated splenic cells stimulated young B/W responding T cells equally. However, old B/W responding T cells failed to be stimulated by young B/W splenic cells. Similar findings were observed in young and old male BXSB mice. Likewise, both female and male splenic stimulatory cells were equally capable of provoking a response in female BXSB responding T cells, but the response of BXSB male T-enriched cells did not improve when exposed to BXSB female stimulating cells. In addition, MRL/l spleen cells stimulated MRL/n responding cells as well as MRL/n spleen cells, whereas MRL/l responding cells were not further stimulated by MRL/n spleen cells. These studies indicate that the stimulating population is behaving normally and that the defect resides within the responding T cell population. Thus, the decreased SMLR suggests the presence of a common T cell alteration shared by these several strains or the presence of differing abnormalities whose common expression is through a selective T cell dysfunction.

The T cell defect in the SMLR does not appear to reflect general

T cell impairment. Diminished SMLR is present in autoimmune mice at a time when the proliferative response to PHA and to alloantigens are still intact. In NZB mice, these mitogenic responses are decreased at approximately 9 months of age (Falkoff et al, 1978), whereas the SMLR is reduced at 6 months. Studies presented in this thesis also indicate that there is no difference in allogenic mixed lymphocyte reaction (MLR) in 4 and 8 month-old B/W mice. In addition, experiments in which the effect of different types of chemical manipulations on SMLR and MLR was examined suggest that the T cell mediating SMLR may be different from the cell responsible for MLR. The results from these experiments indicate that SMLR is more sensitive to X-irradiation, high-dose cyclophosphamide and hydrocortisone than MLR. Further analysis of the data suggests that the precursor cell of the SMLR-responding cell is less resistant to the effect of these chemical manipulations than that of the MLR-responding cell. Hence, the SMLR may be detecting an alteration in a particular T cell subclass.

In old B/W mice, a suppressor activity within the responding population appears not to contribute to the reduced SMLR. Addition of old B/W responding cells to young B/W responding cells did not reduce the syngeneic proliferation of the latter. Furthermore, pretreatment of old B/W responding cells with low-dose X-irradiation did not enhance the SMLR, implying the absence of a radiosensitive suppressor cell which might be inhibiting the SMLR of old B/W responding cells. An abundance of evidence clearly demonstrates that suppressor T cells are relatively radiosensitive prior to activation (Okumura and Tada, 1971; Tada et al, 1971; Dutton, 1973; Eardley and Gershon, 1976). For example, Dutton (1973) has reported that prior to activation by Con A, murine suppressor

T cells for plaque-forming cell responses were highly radiosensitive. Also, in studying the capacity to develop SRBC-specific suppressor T cells in vitro, Eardley and Gershon (1976) showed that suppressor activity was abolished upon exposure to 400 rads X-irradiation in vitro.

What is the nature of this abnormal T cell which is detected by the SMLR assay? Studies designed to characterize the SMLR-responding cell in normal mice were performed in hopes to better understand the nature of the T cell responsible for SMLR. The characterization of the SMLR-responding cell hopefully would be of value in further analyzing the T cell defect present in autoimmune mice. Specific T cell subset surface markers and various physical properties possessed by the SMLR-responding cells were, therefore, examined.

The cell mediating SMLR was demonstrated to bear Lyt1.2 surface antigens. Pretreatment of C57BL/6 responding cell population with monoclonal anti-Lyt1.2 plus rabbit complement abolished most of the proliferative responses towards syngeneic spleen cells. Responding cells pretreated with anti-Lyt2.2 plus complement, however, were still capable of mediating SMLR. Hence, these studies suggest that the SMLR assay is detecting the presence of an abnormal T cell subset expressing the $Lyt1^+$, 23^- phenotype in autoimmune mice.

It is well established that $Lyt1^+$ cells can serve as inducers for various immune effector activities. As indicated earlier, they activate B cells to secrete antibodies and induce precursors of killer cells to mediate killer effector functions. In addition, they play an important role in the immunologic circuits which govern the regulation of immune responses. $Lyt1^+$-T cells are capable of inducing non-immune $Lyt123^+$-T cells to develop feedback inhibitory activity. Recently, abnormalities within immunologic circuits were suggested for three auto-

immune mouse strains, NZB, BXSB, and MRL/l (Cantor and Gershon, 1979). In the NZB, the major T cell lesion is an absence or malfunction of the Lyt123$^+$-T cell mediating feedback inhibition which occurs as early as 1-1/2 months after birth (Cantor et al, 1978). In 3-4 month old MRL/l mice, an inability to suppress Lyt1$^+$ cells has been suggested (Gershon et al, 1978). Other functional defects in Lyt1$^+$-T cells are reported for NZB and B/W mice, but these abnormalities appear with advanced age and disease. As mentioned previously, proliferative response to T cell mitogens and alloantigens mediated by Lyt1$^+$-T cells are severely reduced in old NZB and B/W mice (Leventhal and Talal, 1970; Falkoff et al, 1978). In contrast, the helper Lyt1$^+$ cells involved in antibody response towards T-dependent antigens appear to function normally in both young and old autoimmune mice (Creighton et al, 1979). It is not known whether the Lyt1$^+$-T cell responsible for SMLR is also capable of inducing feedback suppression. But, it is possible that the SMLR assay is detecting similar abnormalities involving Lyt1$^+$-T cells subsets as described in studies which analyzed the feedback inhibitory activities of autoimmune mice.

Prior to the discovery of the Lyt surface antigens, T cell subclasses were mainly distinguished from one another by their differential sensitivities to a variety of chemical manipulations. For example, helper- T cell and suppressor-T cell possess different sensitivities to the effect of X-irradiation and cyclophosphamide. In order to achieve a better understanding on the relationship of the SMLR-responding cell to other T cell subsets, the effect of three well known chemical manipulations on SMLR was investigated. It was observed that the SMLR-responding cell is very sensitive to the effects of X-irradiation, cyclophosphamide and corticosteroids.

It is generally accepted that helper-T cell function is resistant to the effects of ionizing X-irradiation (Katz et al, 1970; Dutton et al, 1971), whereas suppressor-T cell function is relatively radiosensitive prior to activation (Okumura and Tada, 1971; Tada et al, 1971; Dutton, 1973; Eardley and Gershon, 1976). In addition, functional radioresistance among T cells participating in cell-mediated immune reactions has been demonstrated (Möller and Möller, 1965; Asherson and Loewi, 1967; Elves, 1973; Feldman, 1968). For example, Möller and Möller (1965) showed that in vitro killing of target monolayer by non-sensitized mouse lymphocytes was not suppressed by exposing the latter population to 1500 rads in vitro. Also, Feldman (1968) reported that donor lymphocytes exposed in vitro to as much as 6000 rads X-irradiation were still able to passively transfer delayed hypersensitivity. Regarding T cells participating in MLR, it was demonstrated by Sprent et al (1974) that unsensitized alloantigen-reactive T cells were extremely radiosensitive, while alloantigen-activated T cells were found to be highly radioresistant. Analogous to suppressor-T cells and unsensitized alloantigen-reactive T cells, SMLR-responding T cells were also shwon to be quite radiosensitive. In fact, the SMLR-cell appeared to be more sensitive than the MLR-cell to the in vitro effects of X-irradiation.

Observations in several different laboratories strongly suggest the view that one of the important effects of cyclophosphamide administration is the reduction of suppressor-T cells. Early studies of Polak and Turk (1974) first suggested that a short-lived, rapidly proliferating suppressor-T cell, which may be responsible for inhibiting immune response in tolerant animals, was eliminated upon cyclophosphamide treatment. Since then, several laboratories have demonstrated

a differential sensitivity of suppressor-versus-helper function to the effect of cyclophosphamide (Askenase et al, 1975; Debré et al, 1976; Miller et al, 1976). More recently, Cantor et al (1978) reported that the Lyt123$^+$ cells mediating feedback inhibition was sensitive to small doses of cyclophosphamide (10 mg/kg) in vivo. Employing a similar cyclophosphamide treatment protocol as Cantor et al (1978), the effect of cyclophosphamide on SMLR was examined. It was observed that the administration of low-dose cyclophosphamide (20 mg/kg) did not affect SMLR in C57BL/6 mice. However, SMLR was almost completely abolished in mice pretreated with high-dose cyclophosphamide (100 mg/kg) 24 hours prior to the assay. Such pretreatment with high-dose cyclophosphamide slightly decreased MLR. Thus, the SMLR-responding T cell appears to differ from the Lyt123$^+$-T cell mediating feedback suppression in terms of their differential sensitivity to low doses of cyclophosphamide. In addition, the high-dose cyclophosphamide study suggests that the majority of T cells mediating SMLR can be distinguished from those responding in MLR. On the other hand, both suppressor-T cell and SMLR-responding T cell share a common sensitivity to the effects of high-dose cyclophosphamide.

It is well known that cortisone generally suppresses immune function in some species (Kass and Finland, 1953; Berglund, 1956). Based on experiments employing in vivo pretreatment with corticosteroids in mice, cortisone appears to exert differential effects on lymphocytes (Andersson and Blomgren, 1970; Cohen and Claman, 1971). B cells located in the peripheral lymphoid organs are cortisone sensitive, whereas B cell precursors within the bone marrow are cortisone resistant. With regard to

T cells, thymocytes predominantly residing in the thymic cortex and are not fully immunocompetent are cortisone sensitive; the immunocompetent T cells of the thymic medulla and those found in the peripheral lymphoid organs are relatively resistant to the effect of cortisone. Additional studies which examined the relative cortisone sensitivity of T cells in mouse spleen have suggested that peripheral helper-T cells are cortisone sensitive prior to antigen priming but become cortisone resistant following immunization with the carrier. Conversely, GVH reactive cells located in peripheral lymphoid organs are cortisone resistant even prior to antigen stimulation (Segal, et al, 1972). Along similar lines, in this thesis, differential sensitivities to the effect of hydrocortisone are observed in SMLR and MLR in C57BL/6 mice. The results presented indicate that the presence of low concentrations of hydrocortisone succinate ($8x10^{-8}M$) severely reduced SMLR, and slightly decreased the MLR. A similar finding was also reported in humans. Ilfeld et al (1977) demonstrated that the addition of physiological concentrations of hydrocortisone to human cultures caused marked suppression of autologous mixed lymphocyte reaction without any suppression of MLR. Thus, in mice as well as in humans the SMLR (AMLR) and MLR manifest different sensitivities to hydrocortisone. Since the hydrocortisone was added at the beginning of the cultures and remained throughout the culturing period, it cannot be readily predicted which cell type, either the responder or simulator, is being acted upon by the hydrocortisone.

PNA has been used to fractionate thymocytes into two subpopulations: immature and mature cells. Along similar lines, Roelants et al (1979) demonstrated that a small proportion of splenic T cells was agglutinated

by PNA. The splenic cells which were agglutinated by PNA were shown to be comprised of almost equal numbers of $Thy1^+$, Ig^- and $Thy1^-$, Ig^- cells. Thus, splenic PNA^+ cells were postulated to be either of T cell lineage or a null cell. In this dissertation, PNA was utilized to fractionate T-enriched splenic cells isolated from nylon wool into two subpopulations: PNA+ cells and PNA^- cells. The ability of these two subpopulations to respond in SMLR and MLR were then compared. It was predicted that such cell separations with PNA could be used to distinguish the T cell mediating SMLR from that responsible for MLR. Unfortunately, these experiments utilizing PNA failed to distinguish the two responding cells from one another. It was observed that strong SMLR and MLR was displayed by both PNA^+ and PNA^- T cell subpopulations. With regard to SMLR, both PNA^+ and PNA^- cells responded equally to syngeneic spleen cells. Likewise, the PNA^+ cells responded as well as the PNA^- cells towards allogeneic stimulating cells. The above observations suggest that the cells mediating SMLR or MLR are somewhat heterogeneous. PNA separation experiments indicate that at least two subpopulations of T cells, PNA^+ and PNA^- cells exist which are capable of mediating SMLR. MLR reaction also appears to be mediated by at least two different T cell subpopulations. In addition, both SMLR and MLR responding cells share the ability to be agglutinated by PNA. On the other hand, it can be speculated that PNA may not be able to distinguish T cell subpopulations which possess different effector functions. It is possible that the two subpopulations defined by PNA are functionally heterogeneous and overlapping, i.e., PNA^+ as well as PNA^- cells can manifest various T cell functions, and they both can share common T cell functions.

Finally, similar studies using PNA were performed in old B/W male mice. Since at least two T cell subpopulations may be responsible for SMLR as well as MLR, it was thought that a defect in a particular T cell subclass might exist in B/W mice. Such T cell subpopulation defects were not observed in B/W mice. Although the SMLR and MLR in 9-month old B/W mice were poor, both PNA^+ and PNA^- cells of old B/W mice were capable of mediating SMLR and MLR.

The results presented in this study demonstrate that the cell mediating SMLR is a T cell which expresses Lyt1 surface antigens. Like suppressor-T cells, the SMLR-responding cells are extremely sensitive to the effects of high-dose cyclophosphamide and X-irradiation. Moreover, the SMLR-cells appear to differ from the cells mediating MLR in that they possess different sensitivities to exposures with X-irradiation, cyclophosphamide or hydrocortisone. In addition, the SMLR-responding cells may be comprised of heterogeneous subpopulations of T cells. Hence, it is possible that the decreased SMLR occurring in autoimmune mice may reflect abnormalities within several subpopulations of T cells.

The abnormal SMLR in autoimmune mice may be due to the absence or malfunction of a cell or cells mediating SMLR. In the case of a defective cell or cells, the lesion may be either a failure to recognize the stimulating non-T cells or an inability to mediate effector function. Alternatively, a lesion involving another cell type within the responding population can account for the reduced SMLR present in autoimmune mice. Although the role of other cell types in SMLR is unknown, one would predict that like mitogen/antigen-dependent T cell proliferation, T cell

proliferative responses against syngeneic non-T cells may involve a complex series of interaction between different cells. As discussed earlier in the Introduction, biologically active factors such as TCGF or LAF also appear to play prominent roles in the induction of proliferative responses by mitogen/antigen activated T cells. Likewise, the induction of SMLR may involve the generation of TCGF and LAF. Thus, an alteration in a T cell subclass responsible for TCGF production can lead to diminished SMLR in autoimmune mice.

The possibility of a defective TCGF producer occurring in autoimmune mice is (indirectly supported) by a recent study by Gillis et al (1979), which indicated that nude mice manifest abnormal TCGF productions. Gillis and coworkers (1979) provided evidence suggesting that the failure of nude mice to respond in MLC was not due to the lack of alloreactive precursor T cells but to the inability of spleen cells from these athymic mice to produce TCGF. It was observed that lymphocytes from nude mice responded poorly to alloantigens in MLC, whereas the addition of TCGF to MLC led to the restoration of the proliferative as well as the cytotoxic responses to alloantigens. In addition, lymphocytes of nude mice were not capable of producing TCGF upon stimulation with Con A. It then appears that the inability to produce TCGF may contribute to the T cell immunodeficiencies of nude mice.

The question of whether a defect in TCGF production may in part account for the abnormal SMLR observed in autoimmune mice can be resolved in the following ways: (1) by determining whether the addition of TCGF to cocultures of splenic T cells plus syngeneic non-T cells can improve the decreased SMLR present in autoimmune mice; (2) by establishing whether

lymphocytes from autoimmune mice produce decreasing amounts of TCGF upon stimulation with T cell mitogens such as Con A; and (3) if autoimmune mice demonstrate impairment in TCGF production, then the lesion may be either the inability to respond to LAF produced by macrophages or the inability to generate TCGF. This can be partially resolved by determining whether exogeneous LAF can further enhance TCGF production by autoimmune mice. On the other hand, the failure of T cell-TCGF producers to recognize appropriate determinants such as "self Ia" antigens on the stimulating syngeneic non-T cells can also result in diminished TCGF production. If this is the case, then a defect in the ability of a particular cell subclass to be activated by "self Ia" antigens and therefore incapable of producing TCGF may partially contribute to the lowered SMLR in autoimmune mice. Finally, the recognition of "self Ia" determinants by a T cell subclass and subsequent TCGF production occurring during SMLR may provide a mechanism by which immunologic homeostases are maintained.

III. THE STIMULATING CELL in SMLR

In this dissertation, experiments on characterizing the stimulatory cell involved in SMLR were performed. It was observed that spleen cells are strong stimulators of SMLR, whereas bone marrow cells and thymocytes fail to induce a SMLR. Splenic cells which adhere to nylon wool are better stimulators than cells which do not adhere to nylon wool. Therefore, most of the simulatory activities reside within the splenic population which is depleted of T cells. These stimulating cells can also be agglutinated by SBA which provides suggestive evidence that the stimulator is a B cell. Reisner et al (1976b) demonstrated that the cell fraction which is agglutinated by SBA contains 5-8% theta-bearing

cells and 83% IgG or IgM bearing cells and further displays poor response to Con A and PHA but good response to LPS. In addition, splenic cells which are agglutinated by PNA provoke a stronger SMLR than non-agglutinating cells. PNA^+ splenic cells were shown by Roelants et al (1979) to consist of two different cell types: a cell of T cell lineage and a null cell. Since T cells are unable to effectively stimulate other syngeneic T cells, it appears then that another potent stimulator of SMLR is a splenic null cell which can be agglutinated by PNA. Finally, the stimulating population does not seem to depend upon cells which adhere to plastics in that spleen cells depleted of such adherent fractions are still able to stimulate a strong SMLR. These findings indicate that cells which do not adhere well to plastics such as B cells or perhaps null cells can serve as strong stimulators of SMLR. Thus, the indirect evidence presented in this study suggests that the possible cell types within the spleen which are capable of stimulating SMLR are B cells, macrophages or null cells. In the literature, both B cells and macrophages have been reported to strongly stimulate syngeneic T cells (Von Boehmer, 1974; Finke et al, 1976; Glimcher et al, 1980).

IV. AMLR in HUMAN AUTOIMMUNE DISEASE

A proliferative response similar to SMLR occurs in humans when T cells are exposed to autologous non-T cells (Opelz et al, 1975; Kuntz et al, 1976; Hausman and Stobo, 1979). Contradictory findings concerning the nature of the responding cells mediating AMLR are reported. A number of investigators have demonstrated that in humans, the AMLR-responding cell is capable of generating suppressor activity (Innes et al, 1979; Sakane and Green, 1979; Smith and Knowlton, 1979). On the other hand, some investigators have indicated that the responding cell in AMLR can

provide helper functions (Chiorazzi et al, 1979; Hausman and Stobo, 1979). Similarly, divergence of opinions on the nature of the stimulating cell in AMLR also exist. Some laboratories have reported that B cells serve as the most effective stimulators (Opelz et al, 1975; Smith, 1978), whereas others have shown that B cells and monocytes are responsible for the majority of the stimulating activities (Sakane et al, 1978b; Hausman and Stobo, 1979). In addition, one laboratory has indicated that K lymphocytes are the main stimulators in AMLR (Kuntz et al, 1976).

SLE patients, like the autoimmune mice, have decreased AMLR (Sakane et al, 1978b; Kuntz et al, 1979; Sakane et al, 1979). AMLR is also reduced in patients with another autoimmune disorder, Sjögren's syndrome (Miyasaka et al, in press, 1980). Studies which focused on defining the defective cell responsible for the reduced AMLR in SLE patients have generated contradictory findings. In one study, both the responding and stimulating cells from patients with active SLE were shown to behave abnormally in AMLR, while in patients with inactive SLE, only the responding population appeared defective (Sakane et al, 1979). Conversely, a second study concluded that a deficient stimulatory cell accounted for the decreased AMLR occurring in patients with active SLE (Kuntz et al, 1979). Such conflicting observations probably reflect the fact that the results are based on limited studies. In humans, reciprocal cell mixing experiments can only be carried out with SLE patients and other healthy MLR identical siblings.

Diminished suppressor function is present in SLE patients (Abdou et al, 1976; Breshnihan and Jasin, 1977; Horwitz et al, 1977; Sakane et al, 1978a). In addition, Sakane et al (1978b) reported that SLE

patients who had decreased AMLR also failed to generate Con A-induced suppressor T cell activity. These findings imply that defects of both AMLR and suppressor activity may be closely related. In autoimmune mice, a similar correlation between defective SMLR and an abnormal immunological circuit associated with regulation of the immune system is also suggested. Thus, the defects of both AMLR and SMLR and immunoregulatory activities may contribute to the development of autoimmunity in SLE patients and autoimmune mice.

V. COMMENTS on the FUNCTIONAL SIGNIFICANCE of SMLR

The functional significance of SMLR is not known. It may, in part, represent the recognition by T cells against autoreactive B cells or macrophages. If this is the case, defects in SMLR would result in the emergence of autoreactive cells capable of causing autoimmune activities. Alternatively, the SMLR may reflect the various cell interactions occurring in normal immune responses and thus, play a role in maintaining immunological homeostases. This view is indirectly supported by the demonstration that in humans, the AMLR-responding T cells are capable of mediating regulatory functions. Finally, it should be noted that in the SMLR assay, horse sera rather than syngeneic mouse sera was always employed as a nutrient supplement for cell culturing. Hence, the SMLR observed in the experiments presented in this study may not truly represent a response towards syngeneic cells but xenogeneic determinants.

SUMMARY

Murine splenic thymus-derived (T) lymphocytes proliferate when stimulated with syngeneic non-T lymphocytes (SMLR). Several mouse strains genetically susceptible to autoimmune diseases had decreased SMLR compared to normal strains. This decreased SMLR occurred prior to clinical disease and was most pronounced in autoimmune mice which expressed a severe form of the disease. For example, in B/W mice, the SMLR was lower at 7 months than at 2 months of age. The SMLR was lower in BXSB males which develop more accelerated autoimmune disease than in BXSB females. The SMLR was also lower in MRL/l mice than in the related MRL/n mice which develop milder disease. The defect in these autoimmune strains resided within the responding population. Characterization of the SMLR-responding cell in normal mice indicated that it beared the Lytl.2 surface alloantigen and was sensitive to the effects of X-irradiation, cyclophosphamide, and corticosteroid. In addition, it was observed that the responding population of SMLR was comprised of two subpopulations of T cells: a cell which is agglutinated by peanut agglutinin and a non-agglutinating cell. These results suggest that the presence of a common $Lytl^{+}$-T cell defect shared by several autoimmune mice or the presence of other abnormalities whose common expression is through a selective T cell dysfunction.

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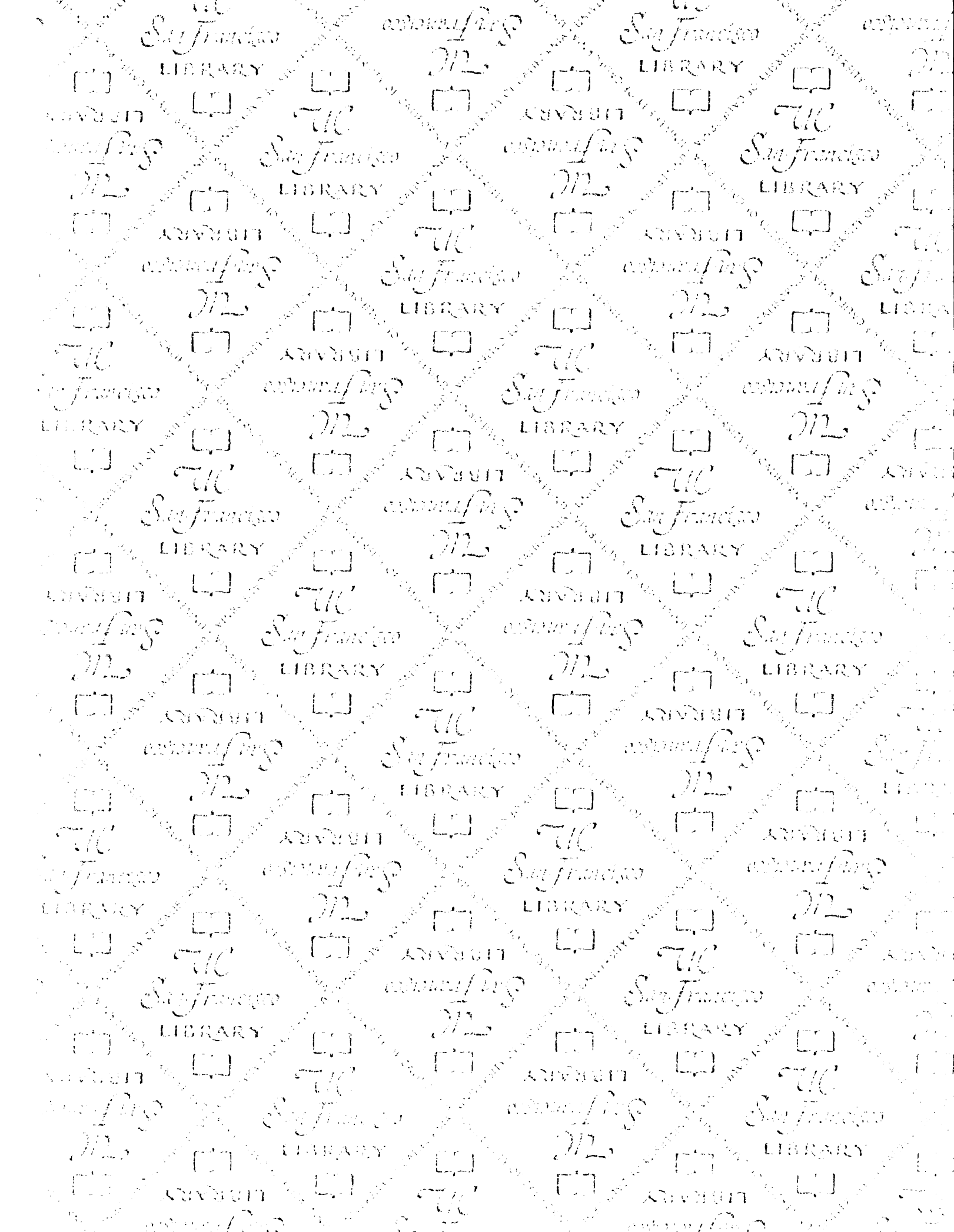
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