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Journal

Journal of Experimental Medicine, 168(6)

ISSN

0022-1007

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Publication Date

1988-12-01

DOI

10.1084/jem.168.6.1979

Peer reviewed

MONOREACTIVE HIGH AFFINITY AND POLYREACTIVE LOW AFFINITY RHEUMATOID FACTORS ARE PRODUCED BY CD5$^+$ B CELLS FROM PATIENTS WITH RHEUMATOID ARTHRITIS

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The CD5 (Leu-1, OKT1) molecule is typically expressed on the surface of human T lymphocytes (1). It is also present, albeit at lower density, on the surface of some human B lymphocytes (2). Recently, we and others (3, 4) showed that CD5$^+$ B lymphocytes constitute a discrete and major subset (~20% of the total circulating or splenic B lymphocytes) within the normal human B cell repertoire. We also showed that the antibodies produced by these cells are polyreactive. They bind to multiple self-antigens such as the Fc fragment of homologous IgG, ssDNA, insulin (Ins)[1], or thyroglobulin (Tg) as well as exogenous antigens, as for example, tetanus toxoid (TT) (3-6). Although these antibodies are polyreactive, they bind to a different extent to various antigens (6, 7). Similarly, in NZB and viable motheaten autoimmune mice, the Ly-1$^+$ B lymphocytes (the mouse equivalent of human CD5$^+$ B cells) make "autoantibodies" that are polyreactive (8, 9).

In patients with rheumatoid arthritis (RA) (4, 10, 11), preliminary experiments suggest that the CD5$^+$ B cell subset may be expanded, but there is little information on the functional state of these cells or the nature of the antibodies produced. In this report, using methods we recently described (3, 7), we determined the number of circulating CD5$^+$ B lymphocytes in patients with RA and normal controls, investigated the functional features of these cells, and studied the reactivity and affinity of the antibodies produced. Our studies show that in RA the CD5$^+$ B cell subset is activated and expanded and produces two types of rheumatoid factor (RF): one that is polyreactive and has low affinity and the other that is monoreactive and has high affinity for Fc fragment of IgG.

Materials and Methods

Preparation of Peripheral Blood B Lymphocytes. Circulating B lymphocytes were obtained from 28 healthy adult subjects (NIH Blood Bank Leukopheresis Research Program) and 13 RA

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[1] *Abbreviations used in this paper:* Ins, human insulin; PE, phycoerythrin; RA, rheumatoid arthritis; RF, rheumatoid factor; Tg, human thyroglobulin; TT, tetanus toxoid.

patients. The RA patients were nine females and four males, age from 21 to 66 yr (mean, 48 yr), all had clinically active, classic seropositive RA, as defined by ARA criteria (12). Serum RF titer ranged from 1:80 to 1:10,240. All patients were receiving nonsteroidal anti-inflammatory drugs at the time of the study; five were receiving prednisone (<10 mg per day), which was not given in the 24 h before obtaining peripheral blood; two were receiving gold salts, and four were receiving low-dose methotrexate weekly. Enriched PBMC fractions were obtained by leukopheresis and fractionated through lymphocyte separating medium (Litton Bionetics, Kensington, MD) (13). T lymphocytes and monocytes were removed as described (13, 14). The remaining mononuclear cell fraction contained 40–50% B cells, <1% T cells, <1% monocytes, and a variable amount of NK cells (3, 13, 14). These cells are referred to here as "enriched B cells."

Flow Cytometry Studies. Enriched B cells (10^6) in 0.1 ml of ice-cold PBS containing 1% BSA (BSA-PBS) were reacted with phycoerythrin (PE)-labeled B1 mouse mAb to CD20 (PE-B1 mAb, IgG2a, Coulter Immunology, Hialeah, FL) or biotinylated mAb to CD5 (anti-Leu-1 mAb, IgG2a, Becton Dickinson & Co., Mountain View, CA) or both reagents. Cell surface receptors for the Fc portions of IgG were blocked using normal human serum (1% final concentration). Cell-bound biotinylated antibody was detected by using FITC-avidin (Sigma Chemical Co., St. Louis, MO) (1.5 μg/10^6 cells). Negative control samples consisted of similar cells reacted with a PE-labeled mAb and a biotinylated mAb of the same isotype (IgG2a, Becton Dickinson & Co.), but with irrelevant specificity, and then with FITC-avidin. The cells were analyzed with a FACS (model 440, Becton Dickinson & Co.) equipped with an argon laser (3, 6). Events (35,000 per sample) were collected and analyzed using a Consort 40 system (Becton Dickinson & Co.). Among red fluorescent ($CD20^+$) cells, those also displaying green fluorescence were designated as $CD5^+$ B cells. Results of one-color analysis were depicted as histograms and two-color analysis data were depicted as contourgrams (14). The threshold of positivity was established on the basis of the analysis of negative control samples. $CD5^+$ and $CD5^-$ B cells were characterized for their forward and 90° light scattering (amplified in a linear fashion), and results were depicted in three-dimensional contourgrams. When required, double-fluorescent ($CD20^+/CD5^+$) and red-fluorescent only ($CD20^+/CD5^-$) cells were gated and sorted as $CD5^+$ and $CD5^-$ B lymphocytes, respectively (3). Total $CD20^+$, including both $CD5^+$ and $CD5^-$, cells were sorted as unfractionated B cells (3). The degree of selectivity achieved in the sorting procedure was assessed as described (3).

Cell Proliferation and Ig Production Studies. Sorted $CD5^+$ and $CD5^-$ B lymphocytes were washed twice with RPMI 1640 medium (Biofluids, Inc., Rockville, MD) containing 10% FCS (Gibco Laboratories, Grand Island, NY), L-glutamine (2 mM), penicillin (50 IU/ml, and streptomycin (50 μg/ml) (FCS-RPMI). These cells were distributed (20,000/well) in quadruplicate cultures in 96-well U-bottomed plates (Nunc, Hazleton, Denver, PA), in the presence or absence of 10^5 syngeneic irradiated (1,800 rad) PBMC as feeder cells, and cultured in 5% CO_2 atmosphere at 37°C. [^{3}H]methylthymidine ([^{3}H]TdR; NEN, Boston, MA, 2μCi/microculture well) was added to different quadruplicate wells on days 1, 2, 3, and 5. On days 2, 3, 4, and 6, fluids and cells were harvested from different quadruplicate sets for Ig titration and [^{3}H]TdR incorporation.

Infection by EBV and Culture of B Cells. Unfractionated, $CD5^+$ or $CD5^-$ B cells were infected with EBV (3, 13, 14) and then distributed at different doses in 96-well round-bottomed microculture plates. The wells contained 10^5 syngeneic or allogeneic irradiated PBMC (previously incubated in FCS-RPMI for 2 h at 37°C and then washed four times with warm FCS-RPMI to remove cytophilic Ig) as feeders. The microcultures were incubated at 37°C in a humidified atmosphere supplemented with 5% CO_2. At the desired time, culture fluids were harvested for the investigation of Ig content and the different antigen-binding activities.

Titration of Ig and Ig with Different Antigen-binding Activities. IgM, IgG, or IgA concentrations were determined using ELISAs similar to those previously described (3, 7, 13) using polystyrene plates (Immulon 2; Dynatech Corp., Alexandria, VA) coated with goat $F(ab')_2$ fragment to human Ig (M + G + A) (Organon Teknika-Cappel, West Chester, PA). Horseradish peroxidase-conjugated affinity-purified $F(ab')_2$ fragments to human μ, γ, or α Ig H chain (Organon Teknika-Cappel) were used as specific probes and the amount of bound horseradish peroxidase-conjugated probe was measured as absorbance at 492 nm using an auto-

mated ELISA reader (Titertek Multiscan Plus; Flow Laboratories, Inc., McLean, VA). Dose-dependent binding curves for IgM, IgG, and IgA were constructed and used as references (3, 13). IgM, IgG, and IgA to various antigens were titrated using antigen-specific ELISAs as described (3, 7, 13). Plates were coated with 5 μg/ml of purified human IgG Fc fragment (52,000 mol wt), purified human IgG_1, IgG_2, IgG_3, IgG_4 (Calbiochem-Behring Corp., San Diego, CA), purified $F(ab')_2$ fragment of human IgG, ssDNA, or purified human Tg, 1.5 μg/ml of purified TT, or 2.5 μg/ml of recombinant human Ins (a gift from Lilly Research Laboratories, Indianapolis, IN), prepared as described (3, 7, 13). Alkaline phosphatase-conjugated affinity-purified goat $F(ab')_2$ fragments to κ and λ L chains (Sigma Chemical Co.) were used to detect Ig L chains. A cocktail of three mouse mAbs to human IgG Fab' fragment (ICN Biomedicals, Inc., Costa Mesa, CA) was used to detect IgG to IgG Fc fragment. Reference binding curves were constructed using antibodies to IgG Fc fragment, ssDNA, Tg, Ins, and TT (13). The lower limit of sensitivity, corresponding to the threshold of linear detectability, of IgM-, IgG-, and IgA-, or antigen-specific ELISAs was 5 ng Ig/ml.

Preparation of Human Monoclonal B Cell Lines and Construction of Cell Hybrids by Somatic Hybridization Techniques. Monoclonal EBV-transformed B cell lines, selected for production of IgG Fc fragment-binding antibodies, were established using sorted $CD5^+$ B cells from RA patients, as described (7). Some of those clones were stabilized by fusion with F3B6 cells, a human-mouse heterohybrid, as described (7). The resulting EBV-transformed B cell hybrids were expanded in selection medium and sequentially cloned at 0.5 cell/well (7). Clones were amplified and the mAbs produced separated as described (7).

Competitive Inhibition Studies. Competitive inhibition studies of the binding of human mAb to solid-phase Fc fragment by the homologous or heterologous soluble ligands were performed as described (7). The dissociation constants (K_d) of the mAbs for IgG Fc fragment were calculated according to Friguet et al. (15). This method has been successfully applied to the calculation of K_d in antigen-antibody systems involving multivalent linkages (7, 16, 17).

Results

Characterization of Peripheral Blood $CD5^+$ B Lymphocytes from Normal Subjects and Patients with RA. The percentage of $CD5^+$ B cells in the peripheral circulation of normal subjects and patients with RA was determined by dual-color fluorescence flow cytometry. Enriched peripheral blood B lymphocytes were reacted with PE-labeled mAb to CD20, biotinylated mAb to CD5, subsequently stained with FITC-avidin, and then applied to the FACS for analysis. Typical contour maps derived are shown in Fig. 1, *A–H*. Evaluation by this method of 28 healthy subjects and 13 patients with RA revealed that 18.0 ± 4.7% (mean value ± SD; range: 9.2–24.4) and 37.4 ± 10.6% (range: 22.1–54.5) of the total B lymphocytes ($CD20^+$ cells), respectively, were $CD5^+$.

$CD20^+/CD5^+$ and $CD20^+/CD5^-$ lymphocytes were then investigated for their light-scattering properties and representative results are depicted in Fig. 1, *I*, *J*, *M*, and *N*. In cytofluorometric studies, the amplitudes of the forward and side light scatter signals are considered proportional to the size and the internal complexity of the cell, respectively (18). Scatter analysis revealed that the $CD5^-$ B lymphocytes were homogeneous and small in both healthy subjects and patients. In contrast, >45% of the $CD5^+$ B lymphocytes from RA patients were large (Fig. 1 *N*) as compared with 6% of the $CD5^+$ B lymphocytes from healthy subjects (Fig. 1 *J*). To measure spontaneous proliferation and Ig secretion, $CD20^+/CD5^+$ and $CD20^+/CD5^-$ B lymphocytes then were sorted and collected as two separate fractions. Reanalysis of the lymphocytes sorted as $CD5^+$ and $CD5^-$ showed that these cells fell back within the coordinates respectively established for the sorting procedure (Fig. 1, *K*,

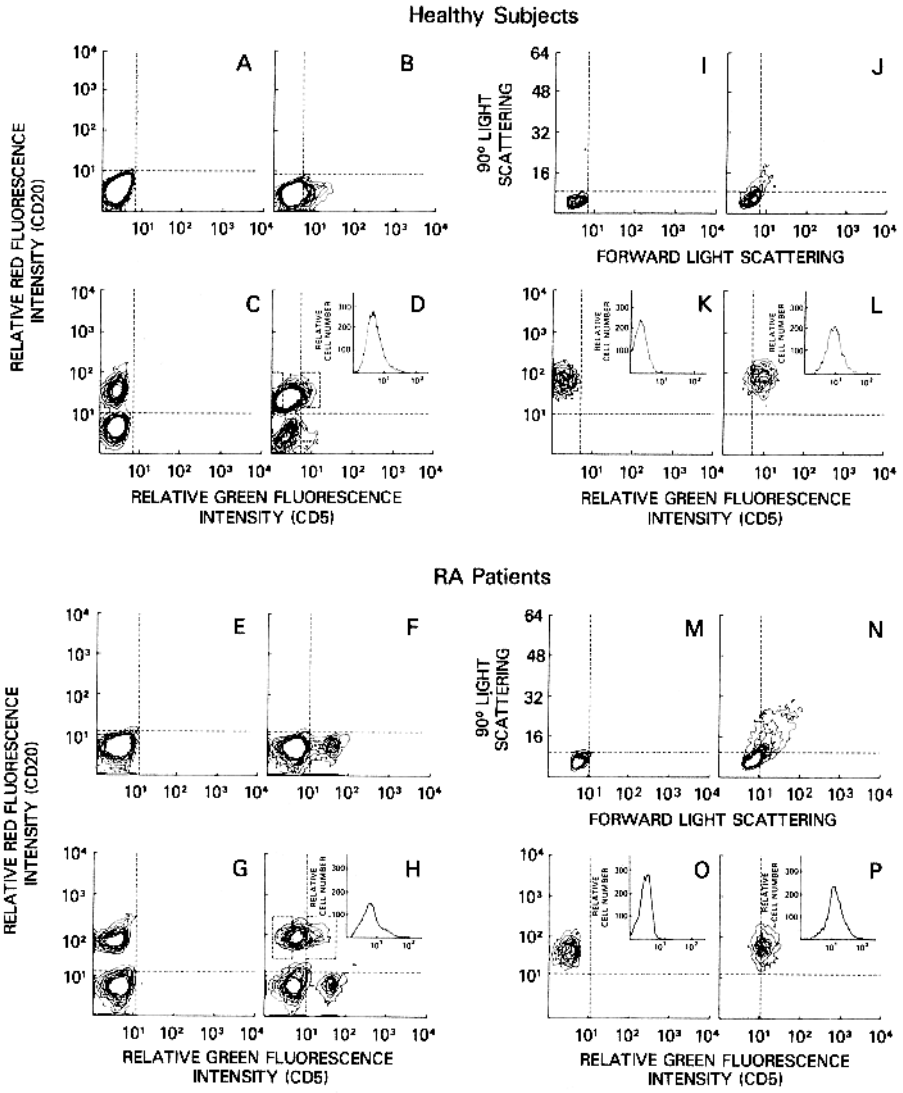

FIGURE 1. Identification, light scattering analysis, and sorting of CD5+ B cells in healthy subjects and RA patients. Data are depicted as computer-derived contourgrams obtained from the cytofluorometric analysis of enriched B lymphocytes. Countourgrams shown here are representative examples of the patterns observed for individuals of the two groups studied. Enriched B lymphocytes from healthy subjects or RA patients were reacted with: biotin-labeled IgG2a mAb and PE-labeled IgG2a mAb (both with irrelevant specificity) and then FITC-avidin (*A* and *E*); biotin-labeled mAb to CD5 (Leu-1) and then FITC-avidin (*B* and *F*); PE-labeled mAb to CD20 (*C* and *G*); biotin-labeled mAb to CD5, PE-labeled mAb to CD20, and then FITC-avidin (*D* and *H*). Double-(red and green) fluorescent ($CD20^+/CD5^+$) cells represent $CD5^+$ B lymphocytes. These cells (*right dotted rectangle*), and their $CD20^+/CD5^-$ counterparts (*left dotted rectangle*), were sorted as $CD5^+$ and $CD5^-$ B lymphocytes, respectively (*D* and *H*). Contourgrams derived from the analysis of the B cells sorted as $CD5^-$ and $CD5^+$ are depicted in *K* and *O*, and *L* and *P*, respectively. Corresponding insets contain the profiles of the relative green fluorescence intensity of these sorted cell fractions (*x*-axis is relative green fluorescence intensity and *y*-axis is relative cell number). Forward (*x*-axis) and side (*y*-axis) light scattering analysis of $CD5^-$ and $CD5^+$ B cells, are shown in *I* and *M*, and *J* and *N*, respectively.

L, *O*, and *P*). The sorted CD5$^+$ and CD5$^-$ B cells then were cultured without added stimulus. Neither CD5$^+$ nor CD5$^-$ B cells from any of the healthy subjects showed significant [^{3}H]TdR incorporation over a 6-d period (Fig. 2 *A*). In contrast, CD5$^+$, but not CD5$^-$, B lymphocytes from each of the patients with RA displayed a high rate of spontaneous [^{3}H]TdR incorporation when cultured under identical conditions (Fig. 2 *B*).

High rates of spontaneous RF production have been reported in culture fluids of circulating PBMC from RA patients (19). To determine whether CD5$^+$ B cells, CD5$^-$ B cells, or both were responsible for this spontaneous Ig secretion, the amount of IgM, IgG, and IgA produced in the microcultures seeded in the above experiment (Fig. 2) was determined. In healthy subjects, neither CD5$^+$ nor CD5$^-$ B lymphocytes spontaneously produced significant amounts of Ig, even after 6 d of culture (Fig. 3, *A–C*). In contrast, unstimulated CD5$^+$, but not CD5$^-$, B cells from patients with RA produced large amounts of IgM, IgG, and IgA (Fig. 3, *D–F*). Moreover, most of the Ig spontaneously produced by CD5$^+$ B cells from RA patients bound to the IgG Fc fragment (not shown).

To further investigate their antibody-producing potential, lymphocytes from 18 healthy subjects and 13 patients with RA were sorted into CD5$^+$ and CD5$^-$ fractions, infected with EBV and cultured at 1,000/well, in presence of irradiated PBMC feeders. After 4 wk, total IgM, IgG, and IgA concentrations were measured in the supernatants of these microcultures. CD5$^+$ B cells from RA patients produced 7,460 ± 578, 162 ± 43, and 66 ± 16 ng/microculture (mean value ± SD of 184 microcultures) of IgM, IgG, and IgA, respectively. These amounts were not significantly different from those produced by CD5$^-$ B cell microcultures from the same subjects (5,953 ± 469, 287 ± 51, and 68 ± 12 ng/microculture, respectively; mean value ± SD of 184 microcultures). Comparable values were obtained when the amounts of IgM, IgG, and IgA were measured in the microcultures seeded with CD5$^+$ or CD5$^-$ B cells from healthy subjects (not shown).

The binding activities of these Ig to TT (an exogenous antigen) and IgG Fc fragment (a self-antigen) are shown in Fig. 4. In both normal controls and patients with

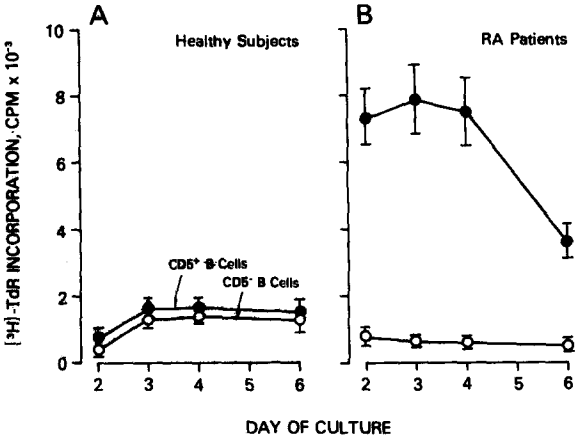

FIGURE 2. Spontaneous proliferation of CD5$^+$ B cells from RA patients. Approximately 2 × 10^4 purified CD5$^+$ (*solid circles*) or CD5$^-$ (*open circles*) B cells from four healthy subjects and five RA patients were seeded in quadruplicate microcultures. [^{3}H]TdR was added on different days and 24 h later the degree of cellular incorporation was measured. Circles are mean values of incorporated [^{3}H]TdR and vertical bars are SE.

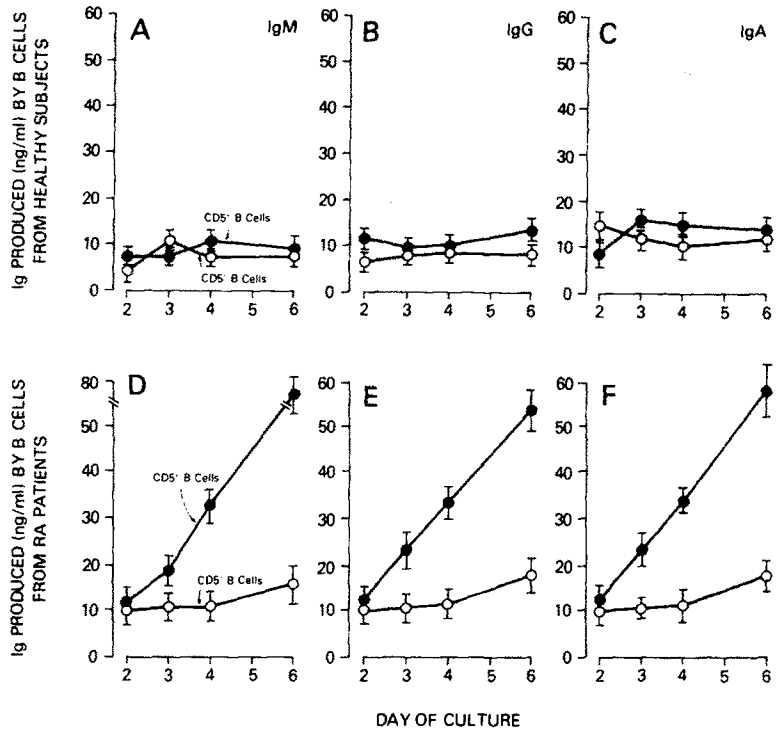

FIGURE 3. Spontaneous Ig secretion by CD5+ B cells fromRA patients. Fluids from microcultures of Fig. 2 were tested for their IgM, IgG, and IgA content. Circles are mean values of the concentrations of IgM, IgG, and IgA produced in 0.2 ml by 2 × 10^4 sorted CD5+ (*solid circles*) or CD5- (*open circles*) B cells from four healthy subjects (*A–C*) and five RA patients (*D–F*). Vertical bars represent SD.

RA, virtually all cells producing antibodies (IgM, IgG, and IgA) to IgG Fc fragment segregated with the CD5+ B lymphocyte subset (Fig. 4 *A*). Cells producing IgM antibodies to TT also segregated primarily with the CD5+ B subset, but cells producing IgG and IgA antibodies to TT segregated with the CD5- B cell subset (Fig. 4 *B*).

Construction of Monoclonal Cell Lines from CD5+ B Lymphocytes and Characterization of the mAbs they Produce. To study the fine specificity of autoantibodies to IgG Fc fragment, EBV-transformed B cell lines were generated using purified CD5+ B cells from three different RA patients. After three sequential cloning steps, 18 EBV-transformed B cell lines producing RF were obtained and the antigen-binding activity of the monoclonal RFs were investigated using ELISAs with a panel of self- and non-self antigens. As shown in Table I, the monoclonal RFs could be divided into two groups based on their antigen-binding reactivity. Eight mAbs (seven IgM and one IgA) were polyreactive; they bound not only to the IgG Fc fragment, but also to the four other antigens tested. The other 10 mAbs (five IgM and five IgA) were monoreactive and bound efficiently only to IgG Fc fragment. In these experiments (Table I), we preferentially selected for B cells producing monoreactive RF.

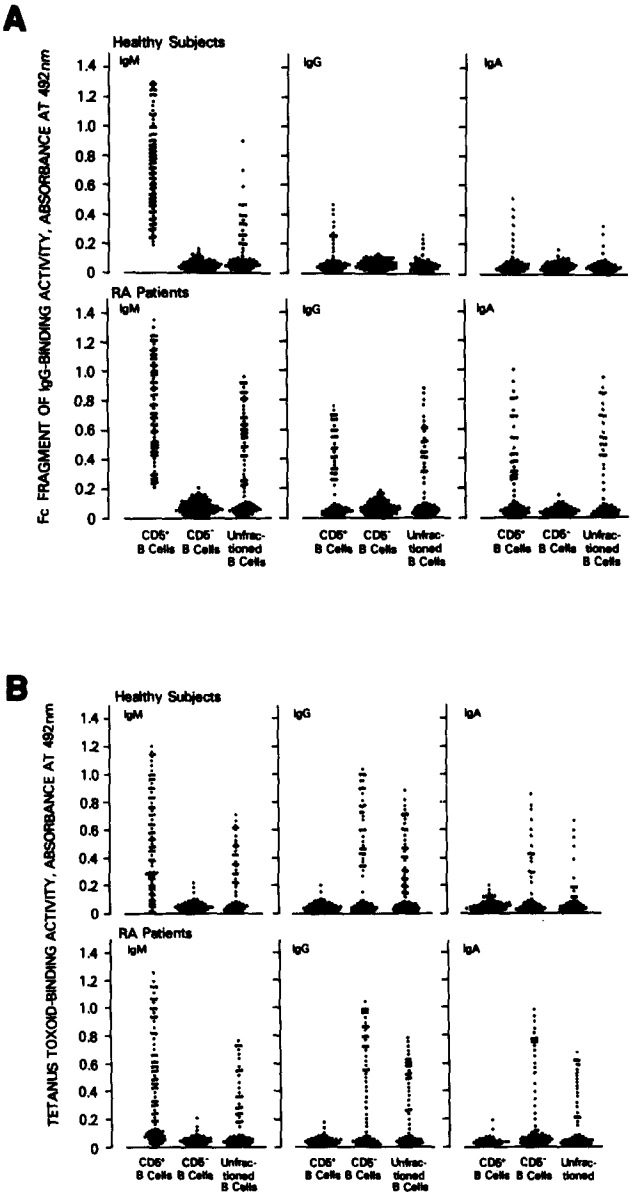

FIGURE 4. Production of IgM, IgG, and IgA antibody to IgG Fc fragment (*A*) or to TT (*B*) by different subsets of B cells. Fluids from microcultures seeded with 1,000 or 2,000 EBV-infected $CD5^+$, $CD5^-$, or unfractionated B cells were tested for the two antigen-binding activities. Each dot represents an individual microculture and ~100 microcultures from each group were tested. Data were derived from two healthy subjects and two RA patients and are representative of the results consistently obtained from these groups of subjects.

In RA, cells committed to the production of monoreactive RF were about one-tenth as frequent as those committed to the production of polyreactive RF. Thus far, in healthy individuals, we could not detect any circulating B cell committed to the production of monoreactive RF.

The multiple antigen-binding capacities of polyreactive RFs were further documented in experiments in which each antibody was tested at different concentrations. All polyreactive RFs bound in a dose-dependent fashion, but to different

TABLE I

Antigen-binding Activity of mAbs Produced by EBV-transformed CD5+ B Lymphocytes from RA Patients

Monoclonal RF‡	H chain	L chain	Antigen-binding activity* Fc	ssDNA	Tg	Ins	TT
274.RA.5.1	μ	κ	0.850	0.450	0.675	0.640	0.254
274.RA.F11§	μ	κ	0.340	0.331	0.344	0.523	0.715
274.RA.31	μ	κ	0.315	0.215	0.720	0.520	0.430
274.RA.14	μ	λ	0.230	0.620	0.240	0.170	0.414
275.RA.2.7	μ	κ	0.540	0.710	0.375	1.076	0.303
291.RA.22.1	μ	λ	0.215	0.223	0.890	0.456	0.159
291.RA.3.6	μ	κ	0.890	0.560	0.785	0.459	0.359
274.RA.5.5	α	κ	0.514	0.155	0.239	0.215	0.317
274.RA.15.1	μ	κ	1.353	0.045	0.038	0.044	0.027
274.RA.F4§	μ	λ	1.098	0.042	0.059	0.037	0.067
274.RA.F5§	μ	κ	1.450	0.049	0.044	0.048	0.055
275.RA.11	μ	κ	1.116	0.037	0.066	0.036	0.045
291.RA.5.2	μ	λ	1.229	0.037	0.047	0.073	0.039
274.RA.F1§	α	κ	1.008	0.071	0.024	0.087	0.041
274.RA.5.3	α	κ	0.987	0.030	0.023	0.045	0.033
274.RA.3.3	α	κ	0.875	0.043	0.054	0.054	0.032
291.RA.7.2	α	λ	1.321	0.077	0.033	0.024	0.043
291.RA.8.7	α	κ	0.779	0.065	0.043	0.032	0.054

* Antigen-binding activity expressed as absorbance at 492 nm. Threshold of positivity was 0.1. Positive values are underscored.

‡ Fc fragment of IgG was used as selecting antigen for the generation of these clones.

§ Monoclonal cell lines stabilized by somatic cell hybridization.

degrees, to each of the antigens tested. The binding curves of three typical polyreactive RFs and three typical monoreactive RFs are shown in Fig. 5, *A–C* and *D–F*, respectively. The specificity of the monoreactive RFs for the IgG Fc fragment was further supported by their inability to bind to the purified $F(ab')_2$ portion of IgG and their comparable ability to bind to the Fc fragment of different IgG subclasses (i.e., IgG1, IgG2, IgG3, and IgG4) (not shown).

The binding activity of polyreactive and monoreactive RFs was studied in greater depth by competitive inhibition experiments (Figure 5, *A'–C'*, and *D'–F'*) in which the binding of a given RF to solid-phase IgG Fc fragment was measured in the presence and absence of soluble homologous (i.e., IgG Fc fragment) or heterologous antigens (i.e., ssDNA, Tg, Ins, or TT). The binding of polyreactive RFs to solid-phase IgG Fc fragment was inhibited in a dose-dependent fashion not only by the soluble Fc fragment itself, but also, and with even higher efficiency, by some of the other soluble ligands tested (Fig. 5, *A'–C'*). The binding of monoreactive RFs to solid-phase IgG Fc fragment also was inhibited in a dose-dependent fashion by the soluble Fc fragment, but not by any of the other soluble heterologous antigens, even at the highest concentrations tested (Fig. 5, *D'–F'*).

The competitive inhibition experiments enabled us to calculate the K_d of RFs for IgG Fc fragment (7, 15). The K_d values of polyreactive RFs were in the range of 10^{-5} mol/liter (Table II). In contrast, the K_d values of monoreactive RFs were ~100

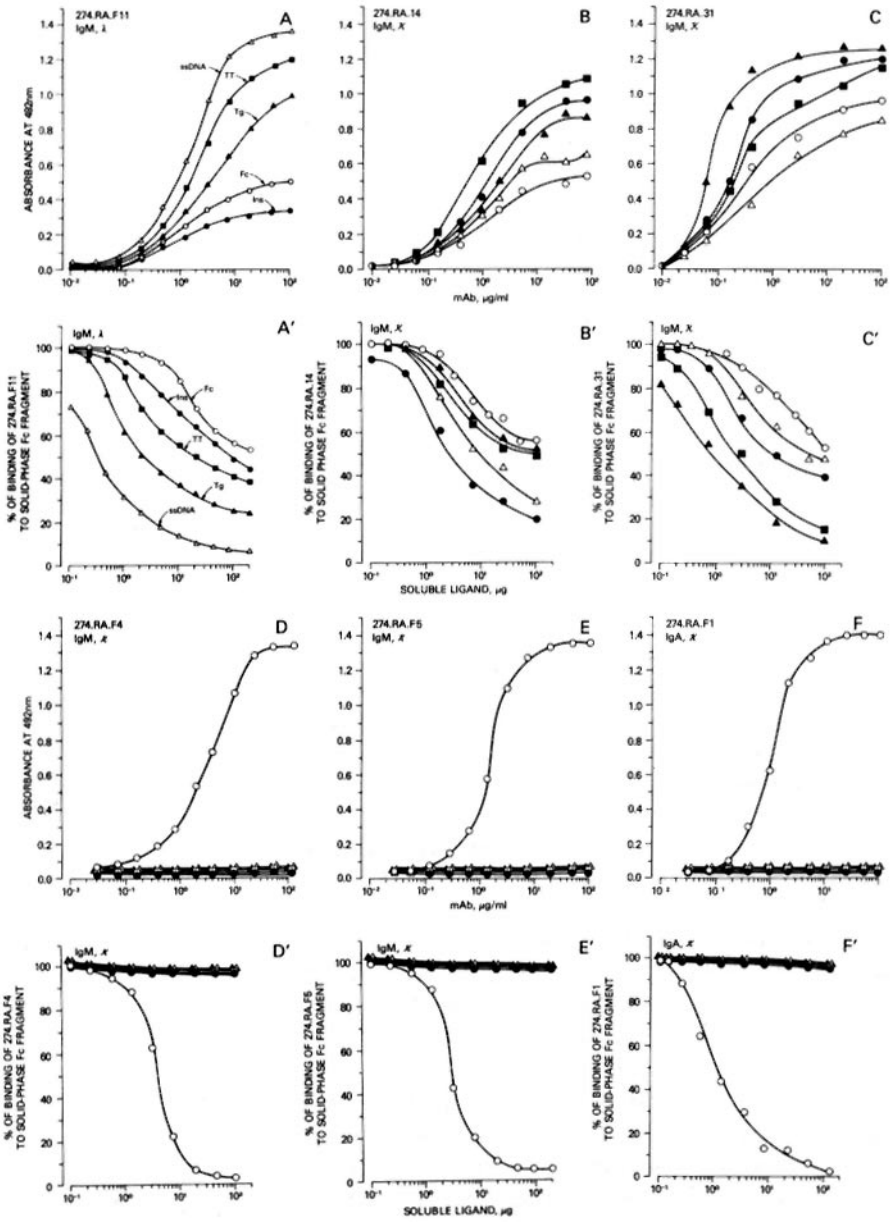

FIGURE 5. Dose-dependent binding of polyreactive and monoreactive RFs (*A–C* and *D–F*, respectively) to solid-phase IgG Fc fragment (○), Ins (●), ssDNA (△), Tg (▲), and TT (■). Dose-dependent inhibition of binding of polyreactive and monoreactive RFs (*A'–C'* and *D'–F'*, respectively) to solid-phase IgG Fc fragment by soluble homologous (Fc fragment) and heterologous (ssDNA, Tg, Ins, or TT) antigens. Samples of each soluble antibody (0.2 μg) were incubated with increased concentrations (0.1–200 μg) of soluble IgG Fc fragment (○), ssDNA (△), Tg (▲), Ins (●), or TT (■). After 18 h, the mixtures were transferred into ELISA plates coated with IgG Fc fragment and the amount of antibody bound to the solid phase was measured. The binding of each antibody in the presence of soluble ligand is expressed as percentage of the binding activity measured after incubation of the same antibody under identical conditions but in the absence of soluble ligand (100% binding activity). This corresponded in each case to at least 0.400 absorbance at 492 nm (range, 0.400–0.800).

TABLE II

Dissociation Constant (K_d, mol/liter) of RFs Generated from CD5+ B Cells of Patients with RA

mAb	H chain	L chain	K_d for IgG Fc fragment
274.RA.F11	μ	λ	4.0×10^{-5}
274.RA.14	μ	κ	3.8×10^{-5}
274.RA.31	μ	κ	2.3×10^{-5}
274.RA.F4	μ	κ	6.0×10^{-7}
274.RA.F5	μ	κ	3.5×10^{-7}
274.RA.F1	α	κ	2.0×10^{-7}

Experimental conditions for the determination of K_d are described in the legend to Fig. 5. The mol wt of IgG Fc fragment was 52,000.

times lower, in the range of 10^{-7} mol/liter. Other polyreactive and monoreactive RFs recently isolated gave similar values (not shown).

Discussion

RFs are autoantibodies directed against determinants on the constant portion of the IgG H chain (20). They are present at high titer in sera and synovial fluids of patients with RA and are thought, but have not been proven, to be relevant to the pathogenesis of this disease. Circulating antibodies (mainly IgM) with RF-binding activity can also be detected in healthy subjects and their titer rises in the course of viral, bacterial, and parasitic infections (21, 22) or during immune response to exogenous antigens (23). Recent studies (3, 4, 6, 7) showed that in healthy subjects virtually all the precursors of the RF-producing B cells belong to the CD5+ B cell subset. Moreover, these RFs are polyreactive and bind to a number of different self (e.g., ssDNA, Ins, Tg, etc.) and non-self (e.g., TT and bacterial LPS) antigens (6, 7).

The present work and recent reports from other laboratories (4, 10, 11) show that the number of CD5+ B cells are increased in patients with RA. In addition, we show here that many of the CD5+, but not the CD5−, B cells from RA patients are activated, spontaneously proliferate, and secrete Ig with RF activity. In contrast, studies in progress (Burastero et al., in preparation.) indicate that CD5+ B cells from patients with SLE are not increased in number and do not spontaneously proliferate in vitro, suggesting that different mechanisms are operative in SLE and RA. Although EBV has been suggested as a possible factor in the pathogenesis of RA (24, 25), immunofluorescence and in situ DNA hybridization on the spontaneously proliferating CD5+ cells has failed to show any evidence of EBV infection (unpublished data).

In addition to the polyreactive RFs produced by CD5+ B cells, the present study shows that some CD5+ B cells from RA patients produce monoreactive high affinity RFs. The dissociation constant of these monoreactive RFs is $\sim 10^{-7}$ mol/liter. In contrast, affinity values (K_{av}) previously reported (26) as characteristic of human RFs ranged between 10^3 and 10^5 liters/mol. This is compatible with the K_d values of our polyreactive RFs from both normal subjects (7) and patients with RA, but lower by a factor of at least 100 as compared with the monoreactive RFs from CD5+ cells of RA patients. Thus, we conclude that in RA patients two types of RFs are

produced: one that is polyreactive and low affinity (K_d, 10^{-5} mol/liter) and the other one that is monoreactive and relatively high affinity (K_d, 10^{-7} mol/liter). The polyreactive low affinity RFs are found in the B cell repertoire of both normal individuals and patients with RA, whereas the monoreactive high affinity RFs have been found, thus far, only in the B cell repertoire of RA patients. Along this line, it has recently been reported that two-thirds of the monoclonal autoantibodies generated from nonimmunized, viable motheaten C57BL/6J autoimmune mice are polyreactive and the remaining one-third are monoreactive (9).

Polyclonal B cell activation has been proposed as a possible mechanism to account for production of autoantibodies in systemic autoimmune diseases (27, 28). Based on this model, one would predict that the autoantibodies produced should have the functional features of the anti-self antibodies found in healthy subject, i.e., polyreactive and relatively low affinity for self-antigens. The present study shows that in patients with RA, polyclonal activation does occur, but is restricted to the $CD5^+$ B cell subset. Moreover, most of the antibodies produced by $CD5^+$ B cells are polyreactive and low affinity, similar to those produced by $CD5^+$ B cells from normal individuals. At variance with this polyclonal activation model are the recent reports by Shlomchik et al. (29, 30), suggesting that in MLR/*lpr* autoimmune mice, a nonrandom antigen-driven clonal selection is operative in generating autoantibodies to IgG Fc fragment and ssDNA. The demonstration in the present study that RFs produced by some of the $CD5^+$ B cells from RA patients are monoreactive and high affinity, would fit with an antigen-driven clonal selection model (31). If this were the case, one would predict that the V_H and V_L gene segments of the monoreactive high affinity antibodies would harbor many more somatic mutations than the V_H and V_L gene segments coding for the polyreactive low affinity antibodies, which appear to be very close to unmutated "germline" genes (20, 32, and Sanz, et al., manuscript submitted for publication). Sequencing the genes of both the monoreactive high affinity and polyreactive low affinity RFs in RA patients, which is now in progress, should help resolve this issue.

It has been suggested that the number of the $CD5^+$ B cells in the circulation of individuals with RA, may be influenced more by the genetic background than by the expression or activity of the disease (33). A scenario that might relate these various observations is that individuals who have high numbers of proliferating $CD5^+$ B cells may be at the greater risk of ultimately developing RA. A high number of proliferating $CD5^+$ B cells would increase the likelihood of occasional cells undergoing somatic mutations (34) and producing monoreactive high affinity RFs (35). These antibodies may be more important in the pathogenesis of RA than polyreactive low affinity antibodies. Along these lines, no direct correlation has been found, thus far, between the titer of RF in the serum of RA patients and the number of circulating $CD5^+$ B cells (unpublished data). Long-term longitudinal studies on the number of $CD5^+$ B cells, their state of activation, and the affinity constants of the RFs they produce would be particularly useful in evaluating the role of $CD5^+$ B cells in the pathogenesis of different stages of RA.

Summary

In patients with rheumatoid arthritis (RA), circulating $CD5^+$ B lymphocytes, but not $CD5^-$ B lymphocytes, are increased in number and size, exist in an activated

state, spontaneously proliferate, and secrete Ig that binds to the Fc fragment of IgG. By constructing continuous mAb-secreting cell lines from $CD5^+$ B lymphocytes, the properties and dissociation constants (K_d) of these antibodies were determined. Two types of rheumatoid factors (RFs) with discrete reactivities were produced. The first type is polyreactive and binds with relatively low affinity (K_d, 10^{-5} mol/liter) to the Fc fragment of IgG. These antibodies are similar to those produced by $CD5^+$ B cells from healthy subjects. The second type of RF is monoreactive and binds with higher affinity (K_d, 10^{-7} mol/liter) to the Fc fragment of IgG. These latter autoantibodies are produced by $CD5^+$ B cells of RA patients, but not healthy subjects. Long-term longitudinal studies are needed to determine the role of these two types of RFs in the pathogenesis of RA.

We are indebted to Dr. J. W. Larrick (Cetus Corp., Emeryville, CA) for providing us with the F3B6 fusion partner hybrid cell line. We thank S. Geis, J. Wheeler, S. Barbieri, and S. Resto for their expert technical help; the personnel of the Leukopheresis Research Program (NIH Blood Bank) and C. Yarboro (Apheresis Unit, NIAMS, NIH) for their collaboration; and E. Mange for secretarial help.

Received for publication 6 June 1988 and in revised form 13 September 1988.

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