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Journal

Journal of Experimental Medicine, 171(1)

ISSN

0022-1007

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Publication Date

1990

DOI

10.1084/jem.171.1.189

Peer reviewed

UNIFORM HIGH FREQUENCY EXPRESSION OF
AUTOANTIBODY-ASSOCIATED CROSSREACTIVE IDIOTYPES
IN THE PRIMARY B CELL FOLLICLES OF
HUMAN FETAL SPLEEN

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Ig H chain V region gene (V_H gene) rearrangement and expression during B cell ontogeny apparently is an ordered and restricted process. In mice there is a developmental bias toward rearrangement and expression of a relatively small subset of Ig V_H genes located most proximal to the J_H locus in the germline DNA (1, 2). Although the relationship between developmental V_H gene expression and V_H gene location is less well understood in man, the repertoire of expressed V_H genes during early B cell ontogeny similarly is restricted, apparently containing only 9–39 genes at 130 d of gestation (3).

Recently, we found that a V_H gene member of this restricted human fetal repertoire frequently is expressed in chronic lymphocytic leukemia (CLL).¹ Previously, we noted that nearly 20% of the CLL patients examined had leukemic cells reactive with a mAb, designated G6 (4). Generated against an IgM rheumatoid factor (RF), this mAb recognizes an Ig H chain-associated crossreactive idiotype (CRI) that is present on many IgM paraproteins with RF activity (5). We found that G6⁺ leukemic cells from unrelated CLL patients express homologous V_H genes that share identity with 51p1 (6), a V_H1 gene estimated to encode ~14% of the antibody H chain V regions in the fetal B cell repertoire (3). These data indicate that the autoantibody-associated G6 CRI is a serologic marker for a conserved and developmentally restricted V_H1 gene that is expressed at high frequency in CLL and early B cell ontogeny.

We have detected expression of other autoantibody-associated CRIs in CLL and related B cell malignancies. Nearly one-fifth of patients with κ L chain-expressing CLL or CD5⁺ small lymphocytic (SL) nonHodgkin's lymphoma (NHL) have neoplastic cells reactive with a murine antiidiotypic mAb, designated 17.109 (7, 8). Generated against an IgM RF from a patient with mixed cryoglobulinemia, this mAb

This work was supported in part by National Institutes of Health grants AR-38475, AG-04100, CA-49870, and AR-25443. T. J. Kipps is a Scholar of the Leukemia Society of America, funded in part by the Scott Helping Hand Fund.

This is publication no. 5978-MEM from the Research Institute of Scripps Clinic.

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¹ *Abbreviations used in this paper:* CLL, chronic lymphocytic leukemia; CRI, crossreactive idiotype; NHL, nonHodgkin's lymphoma; RF, rheumatoid factor; SL, small lymphocytic.

detects a κ L chain-associated CRI present on over one-third of all human IgM RF paraproteins and many other IgM autoantibodies (9, 10). Yet, an additional mAb specific for a distinct RF H chain-associated CRI, named Lc1 (11), labeled neoplastic Ig-expressing B cells from 7 of 56 (13%) patients with CLL or SL NHL (8). Another two patients (4%) in this study were found to have malignant cells reactive with B6, a third mAb specific for an autoantibody H chain-associated CRI that is distinct from either the G6 CRI or Lc1 CRI (8).

Like the G6 CRI, these CRIs may be serologic markers for expression of conserved Ig V genes. 17.109-reactive neoplastic cells from unrelated CLL or SL NHL patients express homologous V_{κ} genes that share 99% homology with *Humkv325* (12, 13), a V_{κ} III gene isolated from placental DNA (14). Similarly, preliminary data indicate that Lc1 and B6 each react with CRIs encoded by a V_H gene(s) of the V_H4 and V_H3 V region subgroups, respectively. As such, these anti-CRI mAbs apparently detect the protein product(s) of conserved Ig V genes that frequently are expressed in CLL and related B cell malignancies.

Because the fetal repertoire apparently shares with CLL the high frequency expression of the V_H1 gene used to encode the G6 CRI, we examined splenic tissue from a 23-wk-old fetus for lymphocytes reactive with G6 and three other mAbs specific for autoantibody-associated CRIs that commonly are detected on the Ig produced in CLL and SL NHL.

Materials and Methods

Tissue Samples. Splenic tissue was obtained from a spontaneously aborted fetus at 161 d of gestation. Blocks of splenic tissue, ~ 1 cm³ in volume, were frozen in optimal cutting temperature embedding medium (Miles Laboratories, Inc., Naperville, IL), using methods described previously (15).

Immunohistochemistry. 4- μ m sections were prepared from the tissue blocks of frozen fetal spleen. Enzyme immunohistochemical staining was performed using an avidin-biotin complex immunoperoxidase technique as described (4, 15). Comparison staining with isotype control murine mAbs was performed at concentrations equal to those used with the specific mAb in each experiment. The percent of stained cells within the primary follicles was determined using photomicrographs of stained primary follicles. For each mAb or mAb combination, a minimum of 100 cells in each of at least five follicles were examined. Statistical calculations of the mean, SD, and student's *t* test of the percent values were performed on a MacIntosh IIcx computer (Apple Computer Co., Cupertino, CA) using Statview II software (Brainpower Inc., Calabasas, CA).

Antibodies. B6, a murine IgG1 mAb generated against the H chain of an IgM RF paraprotein, and G6, a murine IgG1 mAb (5), were provided by Drs. Rizgar Mageed and Roy Jefferis (University of Birmingham, Birmingham, England). An IgG1 mAb specific for a V_{κ} IIIb framework determinant(s) (16), was obtained from Dr. George Abraham (University of Rochester, Rochester, NY). mAb 17.109 is as described (9). Lc1, an IgG1 mAb (11), was the gift of Dr. J. G. P. Sissons (Cambridge University, Cambridge, England). Hybridomas obtained from the American Type Culture Collection (Bethesda, MD) were OKT3, an IgG2a anti-CD3, and DA4-4, an IgG1 antihuman μ H chain. SC-1, an IgG2a mAb reactive with human CD5, was provided by Dr. Robert Fox (Scripps Clinic and Research Foundation). Anti-human κ or λ L chain-producing hybridomas were as described (17). Antibodies were purified from ascites via ammonium sulfate precipitation and protein A-Sepharose column chromatography (Bio-Rad Laboratories, Richmond, CA).

Results

Primary B cell follicles can be identified in the human fetal spleen at 23 wk of gestation. These follicles generally appear as nodular collections of small lympho-

cytes that uniformly bind mAbs specific for human IgM (data not shown). Such follicles typically are contiguous to perivascular clusters of T lymphocytes that bind mAbs to CD3 (Fig. 1, *left*) or CD5 (Fig. 1, *right*). The anti-CD5 mAb labels a larger proportion of lymphocytes than the anti-CD3 mAb, including lymphocytes within the primary follicles (Fig. 1, *arrows*).

We examined photomicrographs of individual follicles from the stained fetal spleen to determine the proportions of follicular lymphocytes that are labeled with a particular mAb or mAb combination. By dividing the number of stained cells by the number of cells within the follicle, we find that 57% ($\pm 3\%$ SD) of the cells within each follicle express κ L chains (Fig. 2, C), and 41% ($\pm 2\%$) of the lymphocytes express λ L chains. Simultaneous staining with both anti- κ and anti- λ mAbs labeled virtually all the cells within each B cell follicle (data not shown).

High proportions of the lymphocytes within each primary follicle were stained with any one of the anti-CRI mAbs (Table I). For any one mAb, there exists little variation between different follicles in the percent number of cells stained. Thus, mAbs specific for antibody H chain-associated CRIs, G6, B6, and Lc1, each stained 6.9% ($\pm 1.7\%$ SD), 5.5% ($\pm 1.0\%$ SD), and 17% ($\pm 0.6\%$ SD), respectively, of the cells within each B cell follicle identified. mAb 17.109, specific for a κ L chain-associated CRI, stained 6.8% ($\pm 0.8\%$) of the follicular lymphocytes.

Simultaneous incubation of the splenic tissue with two or more mAbs allowed us to distinguish overlapping subsets from noncongruous subsets of mAb-reactive lymphocytes. A mAb specific for V_{κ} IIIb framework determinants, for example, stained 9.4% ($\pm 1\%$) of the cells within each follicle (Table I). That 17.109-reactive cells comprised a large subset of such V_{κ} III⁺ lymphocytes was demonstrated in tissue sections that were stained with both antibodies. The percent of the cells reactive to both 17.109 and the anti- V_{κ} IIIb mAbs, 9.3% (± 0.5), was comparable with the proportion of cells labeled by the anti- V_{κ} IIIb mAb alone. However, cells reactive with G6, B6, or Lc1 apparently belong to discrete cell subsets, in that simultaneous staining with any combination of two such anti-CRI mAbs yielded a per-

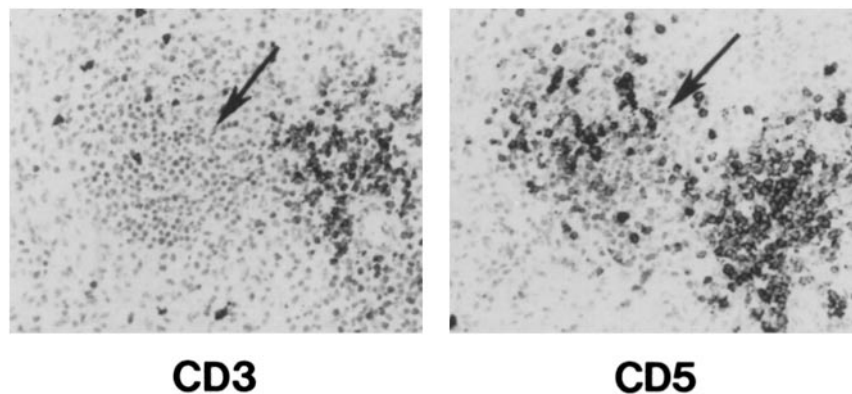


FIGURE 1. Primary lymphocyte follicles in 23-wk-old human fetal spleen ($\times 20$). Contiguous tissue sections were labeled with IgG2a mAbs specific for CD3 (*left*) or CD5 (*right*). Depicted are the same lymphoid aggregates that were identified in the two sections. The primary B cell follicle (*arrow*) lies to the left of a perivascular nodule of T lymphocytes.

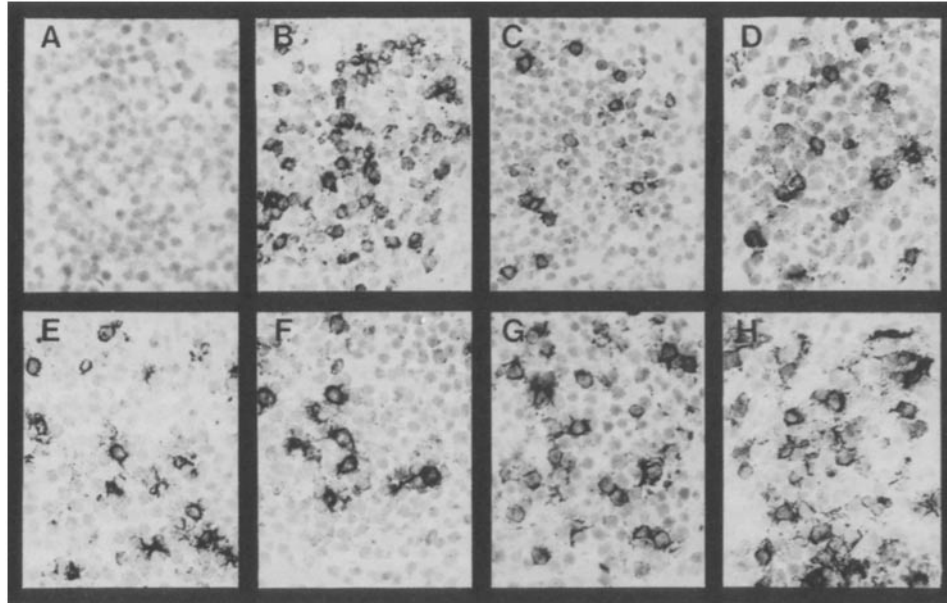


FIGURE 2. Primary B lymphocyte follicles in 23-wk-old human fetal spleen labeled with various mAbs ($\times 40$). (A) Nonspecific mouse IgG1 (MOPC21); (B) anti-human κ L chain; (C) 17.109; (D) anti-V κ IIIb; (E) G6; (F) B6; (G) Lc1; (H) pool of 17.109, G6, B6, and Lc1.

TABLE I
Proportions of Lymphocytes within the Primary Follicles of Fetal Spleen that Are Labeled with Anti-CRI mAbs

mAbs	mAbs				
	17.109	α V κ IIIb	G6	B6	Lc1
	%				
17.109	6.8 \pm 0.6	9.3 \pm 0.6	10.7 \pm 2.2	10.7 \pm 1.2	25.5 \pm 2.3
V κ IIIb	-	9.4 \pm 1.0	ND	ND	ND
G6	-	-	6.9 \pm 1.7	11.6 \pm 1.3	25.8 \pm 2.3
B6	-	-	-	5.5 \pm 1.0	ND
Lc1	-	-	-	-	17.0 \pm 2.2

Percent of lymphocytes within the primary follicles of fetal spleen that are labeled with anti-CRI mAbs (mean \pm SD). Columns and rows are labeled with the mAb used to stain the tissue sections. Identity between column and row labels indicates that only one mAb was used.

cent labeling of follicular cells that was comparable with the sum of the proportions of cells labeled by each mAb separately (Table I). Costaining tissue sections with 17.109 and either B6 or G6 mAbs labeled a smaller percentage of cells than the sum of the percentages of cells labeled by each respective mAb alone ($p = 0.01$ or $p = 0.09$, respectively). These data are consistent with there being a small percentage of cells within the primary follicle that can bind both 17.109 and B6 or 17.109 and G6. Simultaneous staining of the fetal spleen with 17.109, G6, B6, and Lc1 labeled 32.3% ($\pm 1\%$) of all lymphocytes within each B cell follicle.

Discussion

This study indicates that the antibodies produced during early B cell development are comparable with those expressed in CLL. This was inferred from a recent study demonstrating that G6, a major crossreactive idiotype in CLL (4), is encoded by a V_H1 gene that was isolated repeatedly from independent clones of a 130-d fetal liver cDNA library (3, 6). However, this study directly demonstrates that the protein product of this V_H1 gene actually is expressed frequently by disparate cells within human fetal lymphoid tissue. In addition, this study extends on previous work by demonstrating that other CRIs that frequently are expressed in CLL are present on primary follicular B cells. In particular, the κ L chain-associated 17.109 CRI is expressed by $\sim 7\%$ of all cells, and presumably by 12% of κ L chain-bearing cells, within the primary B cell follicles. Leukemic cells bearing the 17.109 CRI invariably have been found to express *Humkv325*, a conserved V_κ gene that apparently is present at single copy in the human haploid genome (12, 13). Thus, our findings indicate that V_κ gene expression by fetal splenocytes is not random, in that *Humkv325* may be expressed by $>10\%$ of all κ L chain-bearing cells within the human fetal spleen. Of note, this is a frequency similar to that noted among leukemic populations from unrelated persons with CLL (7).

That the antibodies produced during early B cell development are comparable with those expressed in CLL or SL NHL may reflect the cell of origin for these B cell malignancies (reviewed in reference 18). Both neoplasms commonly coexpress B cell surface antigens and the 67-kD pan-T lymphocyte surface antigen CD5 (Leu-1). As such, both may be considered neoplasms of the CD5 B cell. Although only a minor lymphocyte subpopulation in the lymphoid organs and peripheral blood of normal adults (18–22), CD5 B cells constitute the predominant B cell subpopulation in human fetal liver and spleen, presumably when the repertoire of expressed V genes still is restricted (23, 24).

This study also indicates that many of the lymphocytes in early human B cell ontogeny express surface Igs that are structurally related to IgM autoantibodies. Although it was originally hypothesized that the restricted expression of human V genes may serve to exclude the appearance of autoantibodies before the emergence of effective T cell immunity (3), studies in the mouse have indicated that many of the antibodies produced in early B cell ontogeny have anti-self reactivity (25). That the antibodies produced by fetal splenic B cells also may have anti-self reactivity is indicated by our finding that the proportion of cells that react with both the 17.109 and G6 mAbs is less than the sum of the proportions of cells that label with each antibody alone. This implies that a fraction of the fetal splenocytes may coexpress both CRIs. Human IgM paraproteins that coexpress both CRIs invariably have been noted to have RF activity (26). Also, the physiologic counterpart to CLL, the CD5 B cell, apparently constitutes a separate B cell lineage that can produce IgM autoantibodies spontaneously in vitro (19, 20, 27–29). Moreover, a high proportion of CLL patients have leukemic cells that express sIg with RF activity (17, 30, 31). Expression of such autoantibodies may be important developmentally, as this may serve to stimulate constitutively early B lymphocytes that have undergone successful Ig V gene rearrangement in the sterile fetal environment.

This study also provides new insight into the physiology of the primary follicle of the human fetal spleen. This is the first study to examine individual B cell follicles

for the proportions of cells that express idiotypic determinants that reflect expression of certain highly conserved Ig V region genes. Previous studies demonstrating restriction in the fetal B cell repertoire (3) could not provide any information on whether individual B cell follicles express all or only part of this restricted repertoire. Conceivably, each B cell follicle could originate from a few, if not one, common clonal ancestors in which successful Ig V_H gene rearrangement had occurred. However, our analyses reveal that each examined follicle had cells that expressed disparate H chain-associated CRIs that presumably are encoded by distinct Ig V_H genes. Moreover, there is little variation between primary follicles in the proportions of cells that express a particular CRI. Finally, costaining with two or more mAbs specific for such CRIs labeled a proportion of cells comparable with the sum of the percentages of cells labeled by each mAb alone. These data indicate that the repertoire of Ig V_H gene rearrangements within any given follicle is heterogeneous. Furthermore, as there exists little fluctuation between follicles in the relative proportions of cells that express any one CRI, the repertoire of Ig V genes expressed in any given follicle may be representative of the entire splenic fetal B cell repertoire. As such, the programmed development of the Ig V gene repertoire apparently is regulated at the level of the individual primary B cell follicle.

Summary

At 23 wk of gestation, the fetal spleen contains follicles of lymphocytes that coexpress B cell differentiation antigens, surface Ig, and the 67-kD pan-T lymphocyte antigen, CD5 (Leu-1). Such cells are thought to represent the normal equivalent cells of B chronic lymphocytic leukemia (CLL). This B cell leukemia is distinctive in that high proportions of patients have leukemic cells that express sIg bearing one or more crossreactive idiotypes (CRIs) that commonly are found on IgM autoantibodies. We performed immunohistochemical studies on fetal spleen at 23 wk of gestation using a panel of mAbs specific for autoantibody-associated CRIs. We find that high proportions (5–17%) of the lymphocytes within each follicle react with any one of the anti-CRI mAbs. Furthermore, there is little variation between primary follicles in the proportions of cells that express a particular CRI. Using a cocktail of four anti-CRI mAbs, we detect autoantibody-associated CRIs on approximately one-third of the lymphocytes within each of the primary B cell follicles. These data indicate that the many of the Igs produced during early B cell development may be structurally related to IgM autoantibodies and to Ig expressed in CLL and related CD5 B cell malignancies. Furthermore, these studies suggest that the repertoire of Ig V genes expressed in each primary B cell follicle may be representative of the total restricted Ig V gene repertoire expressed during early B cell ontogeny.

We thank Drs. Rizgar Mageed and Roy Jefferis (University of Birmingham, Birmingham, England) for the G6 and B6 mAbs, Dr. George Abraham (University of Rochester, Rochester, NY) for the anti-V_κIIIb mAb, and Dr. J. G. P. Sissons (Cambridge University, Cambridge, England) for the Lc1 mAb. We also thank Dr. Kurt Benirschke (Department of Pathology, U.C.S.D. Medical Center, San Diego, CA) for providing us with fetal splenic tissue. In addition, we acknowledge the fine technical assistance of Mr. Grant Meisenholder.

Received for publication 21 July 1989 and in revised form 22 September 1989.

Note added in proof: Stevenson et al. (32) recently reported that some of the lymphocytes in

human fetal spleen express a crossreactive idiotope(s) in common with cold-reactive autoantibodies with anti-I or anti-i binding activity.

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