19. R. S. Schwartz and B. D. Stollar, *J. Clin. Invest.* **75**, 321 (1985).
20. M. Monestier *et al. ibid.* **78**, 753 (1986).
21. Y. Naparsteck *et al.*, *J. Exp. Med.* **164**, 614 (1986).
22. P. P. Chen *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **83**, 8318 (1986).
23. M. P. Madaio, A. Schattner, M. Schattner, R. S. Schwartz, *J. Immunol.* **137**, 2535 (1986).
24. A. J. Manheimer-Lory, M. Monestier, B. Bellon, F. W. Alt, C. B. Bona, *Proc. Natl. Acad. Sci. U.S.A.* **83**, 8293 (1986).
25. We are grateful to B. Perussia for the generous supply of mouse monoclonal antibodies to human mononuclear cell surface determinants, including B36.1, B73.1, and B77.1, and to R. Siraganian for helpful discussions. We thank J. Wheeler, S. A. Barbieri, and S. Geis for their expert technical help, and the personnel of the Leukophoresis Research Program (NIH Blood Bank) for their collaboration. We thank the National Disease Research Interchange, Philadelphia, for promptly making available human spleens and E. Mange for editorial assistance.

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Rheumatoid Factor Secretion from Human Leu-1$^+$ B Cells

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A human B cell subpopulation identifiable by the expression of the cell surface antigen Leu-1 (CD5) is responsible for most of the immunoglobulin M rheumatoid factor secreted in vitro after the cells are stimulated with *Staphylococcus aureus*. The ability of B cells bearing the Leu-1 marker (Leu-1$^+$) to secrete rheumatoid factor is present early in development and extends to adulthood, since Leu-1$^+$ B cells from cord blood and from peripheral blood lymphocytes of both normal adults and patients with certain autoimmune conditions secrete rheumatoid factor in comparable amounts. The neonatal enrichment of Leu-1$^+$ B cells, the presence of Leu-1$^+$ B cells in increased frequencies in patients with autoimmune disease, and the involvement of Leu-1$^+$ B cells in autoantibody secretion suggest both developmental and functional homologies between this human B cell subpopulation and the murine Ly-1 B cell subpopulation.

SINCE THE EARLIEST DAYS OF IMMUNOLOGY much interest has been focused on how the immune system distinguishes between self and foreign antigens and the disturbance of tolerance to self antigens in autoimmune disease. Although we are now beginning to understand many of the mechanisms of the immune system, this long-standing question remains unanswered. Contrary to earlier expectations, B cells reactive with self antigens are not simply deleted, since accumulating data clearly demonstrate that some autoreactive B cells are constituents of the normal B cell population (*1*).

We investigated the possibility that expression of an unrestricted antibody repertoire might be limited to a particular subpopulation of B cells rather than to a "hyperactive" stage of B cells in general. Our earlier work on the murine B cell subpopulation Ly-1 B strongly suggested that these particular B cells include autoantibody specificities. These B cells, which express Ly-1, a pan–T cell glycoprotein, are rare in normal strains of mice but are readily detected in certain autoimmune strains such as NZB and Me^v (*2–4*). Ly-1 B cells sometimes show exclusive proliferation in older NZB mice, and, in fact, the few B cells found in young Me^v mice are mostly Ly-1 B [both strains are known to have high levels of serum autoantibodies of the immunoglobulin M (IgM) isotype]. Ly-1 B cells can be distinguished from other "conventional" B cells phenotypically, physiologically, and functionally. Indeed, our recent studies strongly suggest that these cells may constitute a lineage distinct from B cells that do not express the Ly-1 glycoprotein (Ly-1$^-$ cells) (*5*). One of the most distinctive features of this Ly-1 B cell population is the secretion of large amounts of autoantibodies together with poor response to typical foreign antigens, resulting in a biased response favoring autoantibody specificities (*6*).

The human homolog of Ly-1, known as Leu-1 (OKT1, CD5), is a pan–T cell antigen (*7*) and is also found on a fraction of B cells. Although B cells expressing the Leu-1 antigen (Leu-1$^+$) were first identified as a type of B cell lymphoma (*8*), small numbers of normal cells with this phenotype were detected by immunofluorescence microscopy in restricted anatomical regions (*9*). Further work has shown that these cells constitute a large fraction of B cells early in development (*10, 11*) and also in patients recovering from bone marrow transplantation (*12*). Large numbers of Leu-1$^+$ B cells are found in patients with rheumatoid arthritis (*13*), suggesting a possible functional homology with murine B cells expressing Ly-1.

Analysis of peripheral blood lymphocytes (PBLs) from a group of randomly chosen normal individuals has revealed a relatively constant frequency of B cells (defined as B1$^+$) that express low levels of Leu-1 (CD5) in the absence of other T cell–specific antigens (Leu-2, -3, and -4). Although there is some variation among individuals, Leu-1$^+$ B cells typically constitute 2% to 3% of PBLs and therefore 20% to 30% of total B cells (Figs. 1 and 2). Furthermore, certain individuals in the control group show a distinctive higher frequency of Leu-1$^+$ B cells in the range of 5% to 7% (see Fig. 1). This "high" phenotype is unrelated to the frequency of Leu-1$^-$ B cells and does not represent a general B cell lymphoproliferation. The consistency of the frequency of Leu-1$^+$ B cells in PBLs may suggest that this frequency is controlled by genetic rather than environmental factors.

Investigation of groups of patients with different autoimmune diseases showed an

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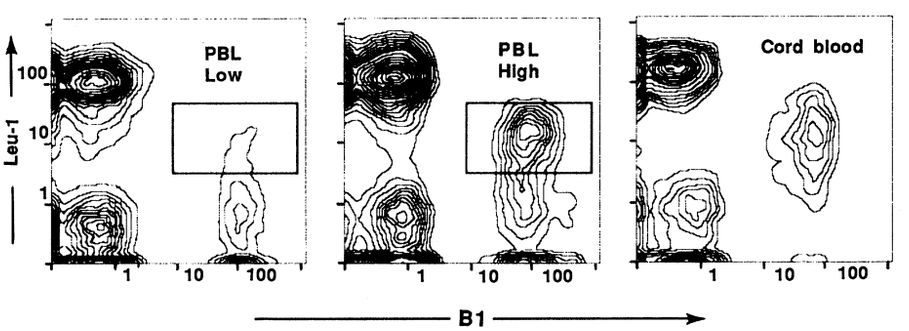

Fig. 1. Peripheral blood of adults contains both high and low phenotypes of Leu-1$^+$ B cells, whereas B cells from cord blood are largely Leu-1$^+$. Ficoll-separated PBLs or cord blood cells were stained with fluorescein-conjugated B1 (Coulter) together with biotin-conjugated antibody to Leu-1 (Becton Dickinson) followed by Texas red–avidin in a second incubation and then analyzed on a dual-laser FACS 440 interfaced with a VAX-11/730 computer (*2*). The Leu-1$^+$ B region is boxed. Contour lines are drawn to enclose equal percentages of cells.

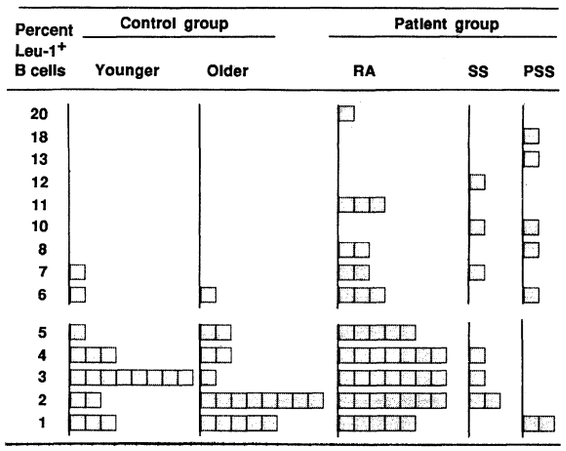

Fig. 2. Groups of patients with certain autoimmune diseases include increased numbers of individuals with a high Leu-1$^+$ B cell phenotype. Peripheral blood lymphocytes were stained and analyzed as in Fig. 1. Frequencies are among cells with lymphoid forward and large angle scatter (eliminating monocytes). Younger controls are predominantly 20- to 30-year-old males. Older controls are 50- to 60-year-old females with no autoimmune symptoms. RA, Rheumatoid arthritis; SS, Sjögren's syndrome; PSS, progressive systemic sclerosis.

increased frequency of individuals with the "high" Leu-1$^+$ B cell phenotype (over 5%) (Fig. 2). In fact, 25% of rheumatoid arthritis (RA) patients have this phenotype compared to 10% of individuals in a control group. In addition, some patients have unusually high levels of Leu-1$^+$ B cells (up to 20%) compared with those found in the normal control group. However, increased numbers of Leu-1$^-$ B cells are also often detected in these same patients (20% or more), possibly reflecting a general B cell proliferation not limited to Leu-1$^+$ B cells. Similar analyses of smaller groups of patients with Sjögren's syndrome and progressive systemic sclerosis also suggest a corresponding increased frequency of individuals with high levels of Leu-1$^+$ B cells.

Leu-1$^+$ B cells from any of these individuals express IgM, IgD, HLA-DR, and other pan–B cell antigens (such as B1 and B2). However, the expression of activation antigens such as Ba (*14*), transferrin receptor, or interleukin-2 receptor are not associated significantly with the Leu-1$^+$ B cell population, even in patients with high levels of these cells. This fact, together with the constant frequency described above, suggests that Leu-1$^+$ B cells represent a relatively independent B cell subpopulation rather than simply an activated form of Leu-1$^-$ B cells. Analysis of the appearance of B cells during ontogeny supports this view and shows remarkable similarity with murine Ly-1 B. That is, a large fraction (75%) of the B cells in cord blood are Leu-1$^+$ in marked contrast with adult PBLs in which Leu-1$^+$ B cells typically constitute a minor population (Fig. 1). This is consistent with previous reports of enriched frequencies of Leu-1$^+$ B cells in fetal spleen and corresponds well with the early appearance of murine Ly-1 B as major B cell population in early development.

A comparison of the functional activity of murine Ly-1$^+$ B cells with human Leu-1$^+$ B cells demonstrates further homology between these two populations. Although we have found no correlation between levels of Leu-1$^+$ B cells and grade of clinical symptoms or level of serum immunoglobulins, we have observed substantial enrichment of rheumatoid factor (RF) secretion from the Leu-1$^+$ B cell population compared with other B cells after in vitro stimulation with *Staphylococcus aureus* Cowan I cells (SAC) (*15, 16*). This experiment was performed by using fluorescence-activated cell sorting (FACS) to deplete from PBLs either the Leu-1$^+$, B1$^+$ (Leu-1$^+$ B) or the Leu-1$^-$, B1$^+$ (Leu-1$^-$ B) cell fractions (leaving T cells and other B1$^-$ cells unchanged in both sorted fractions). Supernatants from such fractions cultured in the presence or absence of SAC were then assayed (after 7 days) by an enzyme-linked immunosorbent assay (ELISA) for total IgM and for RF (IgM antibody to human IgG).

We detected very little spontaneous IgM secretion from the unstimulated cultured fraction enriched in Leu-1$^+$ B cells (Table 1). Although this may partly reflect the paucity of B cells in the Leu-1$^+$ B cell fraction (that is, PBLs depleted of Leu-1$^-$ B cells), similar results were also obtained when equal numbers of positively sorted B cell fractions were cultured. In contrast, after SAC stimulation we were able to detect large amounts of RF in the fraction enriched in Leu-1$^+$ B cells, and much less in the fraction depleted of Leu-1$^+$ B cells. Secretion of IgM could be induced by either a low (0.001%) or a high (0.01%) dose of SAC. However, RF secretion was significantly greater at the higher SAC dose even though total IgM secretion was often less. With the high SAC concentration we found that much higher levels of RF were secreted by the Leu-1$^+$ fraction than by the Leu-1$^-$ fraction (Table 1 and Fig. 3). In contrast, almost comparable levels of total IgM secretion were observed in supernatants from either fraction (although often slightly higher at the 0.01% SAC dose from the Leu-1$^+$ B cell fraction). This enrichment for RF secretion in total IgM from the Leu-1$^+$ B cell fraction (Fig. 3) was not limited to cells sorted from patients with autoimmune disease, as comparable results were obtained

Table 1. Induced RF comes primarily from the Leu-1$^+$ B cell fraction. Cells sorted from RA patients, normal controls, or cord blood (CB) (depleted as described in the text) were cultured for 7 days with or without 0.01% SAC and then assayed for RF and total IgM. Values shown are units of RF secretion (1 U = 10^{-5} dilution of a high RF-containing serum) and of total IgM secretion. Levels of secretion were unaffected by incubation with staining reagents. Reanalysis of sorted fractions showed >95% depletion of the appropriate cell type (Leu-1$^+$ B or Leu-1$^-$ B).

Individual	Type	Leu-1$^+$ B-containing fraction				Leu-1$^-$ B-containing fraction			
		Unstimulated		SAC-stimulated		Unstimulated		SAC-stimulated	
		RF (U)	Total IgM (ng/ml)	RF (U)	Total IgM (ng/ml)	RF (U)	Total IgM (ng/ml)	RF (U)	Total IgM (ng/ml)
1	RA	<8	39	203	1,932	<8	293	13	3,605
2	RA	<8	13	366	3,732	<8	41	72	5,464
3	RA	<2	30	152	5,100	<2	110	3	2,650
4	Control	<2	28	95	4,750	<2	110	<2	163
5	Control	<2	<15	98	6,700	<2	18	<2	2,300
6	Control	<1	75	134	14,350	<1	290	21	11,650
	CB	<2	11	12	25	<2	<6	<2	<6
	CB	<2	<6	28	31	<2	<6	<2	<6
	CB	<3	117	210	5,175	<3	63	77	6,155

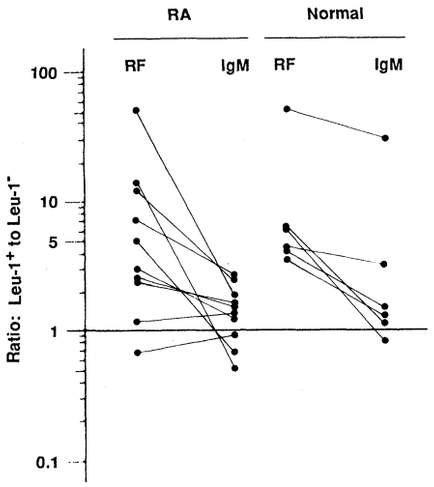

Fig. 3. Leu-1$^+$ B cells secrete a larger amount of RF than Leu-1$^-$ cells but secrete similar amounts of IgM. Data are the ratio of amounts secreted by Leu-1$^+$ B cells to that secreted by Leu-1$^-$ B cells from ten patients with rheumatoid arthritis and six control individuals determined as described in Table 1. Corresponding values from the same individual are connected by lines.

with cord blood and with PBLs from normal individuals [see also (*16*)].

Since our sorting experiments relied on depleting Leu-1$^+$ or Leu-1$^-$ cells that express B1, we verified (by depleting total B1$^+$ cells) that most of this SAC-induced RF secretion is derived from cells initially expressing the B1 (BP35, CD20) molecule. Therefore, our observations imply that RF secretion is derived from Leu-1$^+$ B cells even in cases where very few of these cells are present and that they are capable of high levels of RF secretion. Small amounts of secretion occasionally observed in a B1$^-$ fraction presumably resulted from preexisting terminally differentiated secreting cells (*17*). There remained the possibility that the enriched RF secretion observed in the Leu-1$^+$ fraction was caused by binding of the anti-Leu-1 staining reagent to B cell Fc receptors or to surface Ig (since RF cells are specific for IgG). However, in similar depletion-sorting experiments with mouse IgG2a control reagents unreactive with any human antigens, no significant enrichment (or depletion) in RF secretion was observed.

Our findings of enriched secretion of RF by a subpopulation of B cells has already been foreshadowed through fractionation of B cells by binding to mouse erythrocytes. Human B cells binding to mouse erythrocytes can be induced to secrete rheumatoid factor after Epstein-Barr virus stimulation (*18*). Moreover, chronic lymphocytic leukemia cells (which express Leu-1) also rosette with mouse erythrocytes (*19*), an indication that Leu-1$^+$ B cells may have been the source of RF in this earlier study. However, preliminary observations show that although such rosetting does enrich for Leu-1$^+$ B cells, it does not yield a pure population and therefore cannot be conclusive in assigning a functional role to these cells.

Our observations demonstrate that under the conditions we used, a particular subpopulation of B cells, which appears to be a separate lineage from Leu-1$^-$ B cells, is largely responsible for RF secretion. Although the significance of the increase in Leu-1$^+$ (or Ly-1$^+$) B cells in certain autoimmune diseases remains to be resolved, we might speculate that triggering to RF secretion would occur more readily in individuals with high levels of Leu-1$^+$ B cells, resulting in a predisposition to certain types of autoimmune diseases such as rheumatoid arthritis, Sjögren's syndrome, and progressive systemic sclerosis. It is intriguing that early in development, cells with features identical to this "autoimmune" population constitute a major fraction of B cells. The presence of a similar set of B cells in such a phylogenetically divergent species as the mouse suggests that these cells may have an important functional role and may provide a key to understanding the mechanism of antibody diversity in both mouse and man.

REFERENCES AND NOTES

1. L. Hang *et al.*, *J. Exp. Med.* **157**, 874 (1983).
2. K. Hayakawa *et al.*, *ibid.*, p. 202.
3. V. Manohar, E. Brown, W. M. Leiserson, T. M. Chused, *J. Immunol.* **129**, 532 (1982).
4. C. L. Sidman, L. D. Shultz, R. R. Hardy, K. Hayakawa, L. A. Herzenberg, *Science* **232**, 1423 (1986).
5. K. Hayakawa, R. R. Hardy, L. A. Herzenberg, L. A. Herzenberg, *J. Exp. Med.* **161**, 1554 (1985).
6. K. Hayakawa *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **81**, 2494 (1984).
7. L. A. Ledbetter *et al.*, *J. Exp. Med.* **153**, 310 (1981).
8. C. Y. Wang, R. A. Good, P. Ammirati, G. Dymbort, R. L. Evans, *ibid.* **151**, 1539 (1980).
9. F. Caligaris-Cappio, M. Gobbi, M. Bofill, G. Janossy, *ibid.* **155**, 623 (1982).
10. M. Bofill *et al.*, *J. Immunol.* **134**, 1531 (1985).
11. J. H. Antin *et al.*, *ibid.* **136**, 505 (1986).
12. K. A. Ault *et al.*, *J. Exp. Med.* **161**, 1483 (1985).
13. C. Plater-Zyberk, R. N. Maini, K. Lam, T. D. Kennedy, G. Janossy, *Arthritis Rheum.* **28**, 971 (1985).
14. H. Kikutani *et al.*, *J. Immunol.* **136**, 4019 (1986).
15. A. Forsgren, A. Svedjelund, H. Wigzell, *Eur. J. Immunol.* **6**, 207 (1976).
16. A. I. Levinson previously reported stimulation of RF secretion by SAC from both PBL and cord blood cells (*Proceedings of the 6th International Congress of Immunology*, Toronto, Canada, 6 to 11 July 1986, abstract 3.44, p. 22).
17. A. W. Boyd *et al.*, *J. Immunol.* **134**, 1516 (1985).
18. S. Fong, J. H. Vaughan, D. A. Carson, *ibid.* **130**, 162 (1983).
19. G. Stathopoulos and E. V. Elliott, *Lancet* **1974-I**, 600 (1974).

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Identification of Human Uromodulin as the Tamm-Horsfall Urinary Glycoprotein

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The primary structure of human uromodulin, a 616–amino acid, 85-kilodalton glycoprotein with in vitro immunosuppressive properties, was determined through isolation and characterization of complementary DNA and genomic clones. The amino acid sequence encoded by one of the exons of the uromodulin gene has homology to the low-density-lipoprotein receptor and the epidermal growth factor precursor. Northern hybridization analyses demonstrate that uromodulin is synthesized by the kidney. Evidence is provided that uromodulin is identical to the previously characterized Tamm-Horsfall glycoprotein, the most abundant protein in normal human urine.

UROMODULIN IS AN 85-KD GLYCOprotein isolated from the urine of pregnant women that is reported to be a potent immunosuppressive molecule (*1*). It inhibits antigen-induced T-cell proliferation and monocyte cytotoxicity in vitro at concentrations as low as 30 p*M* (*1*). Uromodulin has also been shown to be a high-affinity ligand for interleukin-1 (IL-1); it specifically inhibits a mouse thymocyte comitogenic assay dependent on IL-1 (*2*). The immunosuppressive activities of uromodulin are reported to reside predominantly in the N-linked carbohydrate residues of the protein (*2, 3*). Preliminary characterization of the purified protein revealed a very high carbohydrate content (~30%), a tendency to form aggregates, and the presence of intrachain disulfide linkages (*1*).

Another 85-kD polypeptide known as the Tamm-Horsfall glycoprotein (*4*) has many physical and biological characteristics in common with uromodulin. The Tamm-Horsfall glycoprotein is the most abundant protein of renal origin in normal urine and contains approximately 30% carbohydrate (*5*). The Tamm-Horsfall glycoprotein has also been found to have immunosuppressive

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