iting avascular necrosis and those of the *Clidastes* material that did not. Exposure to a radiation source is an interesting possibility because radiation bombardment is one suggested cause for Cretaceous extinctions (*13*). This probably would not be recognizable as an increase in bone radioactivity, but should have affected *Clidastes* also, and might be sought in other contemporary animals.

It is also known that bismuth poisoning can cause avascular necrosis (*14*); electron-probe analysis of some of the affected vertebrae failed to yield any significant concentrations. It is possible, however, that the diets of *Tylosaurus* and *Platecarpus* included organisms that concentrated some similarly toxic elements.

The most likely explanation for the high frequency of avascular necrosis in mosasaurs is caisson disease or the bends. Although whales apparently do not suffer bends, their anatomical modifications may not have been present in the mosasaurs. Young reported that whales store extra oxygen in the retea mirabile, an extensive blood vessel network (*15*). The high frequency of avascular necrosis in mosasaurs suggests that they lacked such an intravascular oxygen storage system. If such an oxygen-storage system was a later evolutionary event, the oxygen-storage system for mosasaurs may have been the air spaces of the lungs. This might have resulted in the equivalent of the scuba apparatus–associated phenomenon of avascular necrosis. The implications for mosasaurs transcend the bony phenomenon noted; the manifestations of avascular necrosis in man include symptomatology such as cerebrovascular accidents, which are detrimental to the organism's survival.

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16. We wish to thank O. Bonner and J. D. Stewart for sharing their extensive knowledge of the Niobrara Chalk fauna and for reviewing the manuscript. E. Zeller and G. Dreschoff made radiological tests on the University of Kansas Mosasaur Collections. W. Duellman and L. Trueb critically read the manuscript.

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Human Lymphocytes Making Rheumatoid Factor and Antibody to ssDNA Belong to Leu-1$^+$ B-Cell Subset

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B lymphocytes bearing the Leu-1 cell-surface antigen (Leu-1$^+$), the human equivalent of mouse Ly-1$^+$ B lymphocytes, have been detected in human peripheral blood, but there is little information on their frequency and properties. Analysis by fluorescence-activated cell sorter and double immunofluorescence showed that Leu-1$^+$ B cells are consistently present in the peripheral blood and spleens of healthy subjects and constitute 17.0 ± 5.0% (mean value ± standard deviation) and 17.3 ± 3.9%, respectively, of total B cells. When purified Leu-1$^+$ and Leu-1$^-$ B lymphocytes were transformed into immunoglobulin-secreting cells by infection with Epstein-Barr virus and the culture fluids were tested for reactivity with self-antigens, at least two important autoantibodies, antibody to the Fc fragment of human immunoglobulin G (rheumatoid factor) and antibody to single-stranded DNA, were found to be made exclusively by Leu-1$^+$ B cells. It is concluded that the Leu-1$^+$ lymphocytes represent a major subset of the normal human B cell repertoire and include the B cells capable of making autoantibodies similar to those found in systemic lupus erythematosus and rheumatoid arthritis.

THE LY-1 MARKER IS TYPICALLY EXpressed on the surface of all mouse T lymphocytes (Thy-1$^+$), including the helper and the suppressor/cytotoxic T cell subsets (*1*). The Ly-1 marker is also expressed on the surface of a minor subset of normal mouse B lymphocytes, albeit at a low density (*2–4*). In autoimmune NZB mice, this Ly-1$^+$ B cell subset is expanded and is responsible for the immunoglobulin M (IgM) "spontaneously" secreted in vitro by spleen cells (*3, 5*). These IgM include autoantibodies that react with single-stranded DNA (ssDNA) and thymocytes (*5*).

A fraction of human B lymphocytes from adult peripheral blood, lymph nodes, and tonsils were shown to express at low density the surface Leu-1 (CD5) molecule, the human equivalent of the mouse Ly-1 molecule (*6–10*). We have now used specific mouse monoclonal antibodies to Leu-1 and to human B lymphocytes in double-fluorescence flow cytometry to identify and quantitate Leu-1$^+$ B lymphocytes in peripheral blood and spleens from healthy adult subjects. Using in part a methodology we recently described (*11*), we separated Leu-1$^+$ B cells from their Leu-1$^-$ counterparts by cell sorting and infected the cells with Epstein-Barr virus (EBV), which transforms B lymphocytes into immunoglobulin (Ig)-secreting cells. Culture fluids were then investigated for Ig content and reactivity. The lymphocytes capable of producing autoantibodies similar to those found in at least two important human autoimmune diseases, systemic lupus erythematosus (SLE) and rheumatoid arthritis (RA), consistently segegated with the Leu-1$^+$ B cell subset.

The first set of experiments was designed to identify and quantitate Leu-1$^+$ B lymphocytes in healthy subjects. A mononuclear cell fraction enriched in B cells was prepared from human peripheral blood (*12*) and treated with phycoerythrin-conjugated mouse monoclonal antibody (PE-mAb, IgG2a) to B1 (CD20, a B cell marker) and biotin-labeled mAb (biot-mAb, IgG2a) to Leu-1. The cells were washed, incubated with fluorescein-conjugated avidin (FITC-avidin), washed again, and analyzed by fluorescence-activated cell sorter (FACS) (Fig. 1) for the presence of B lymphocytes bearing the Leu-1 marker. Figure 1D shows that approximately 14.0% of B1$^+$ cells (red fluorescence) also displayed the Leu-1 marker (green fluorescence). Leu-1 was expressed at low density on the surface of these B lymphocytes as indicated by their "dim" green fluorescence (cells within rectangle, upper right quadrant of Fig. 1D) as compared with the stronger fluorescence intensity of the residual T cells (Fig. 1D, lower right quadrant) also present in the material submitted for analysis. By this procedure, the percentage of Leu-1$^+$ B lymphocytes in the peripheral blood of 18 healthy subjects was measured. It was found that 9.0% to 26.6% (mean ± SD, 17.0 ± 5.0%) of B cells were Leu-1$^+$. Similarly, Leu-1$^+$ B cells represented 15.3% to 23.6% (17.3 ± 3.9%) of the B lymphocytes recovered from the spleen of six adult subjects. In other experiments, similar percentages of Leu-1$^+$ B lymphocytes were detected with different mAbs to

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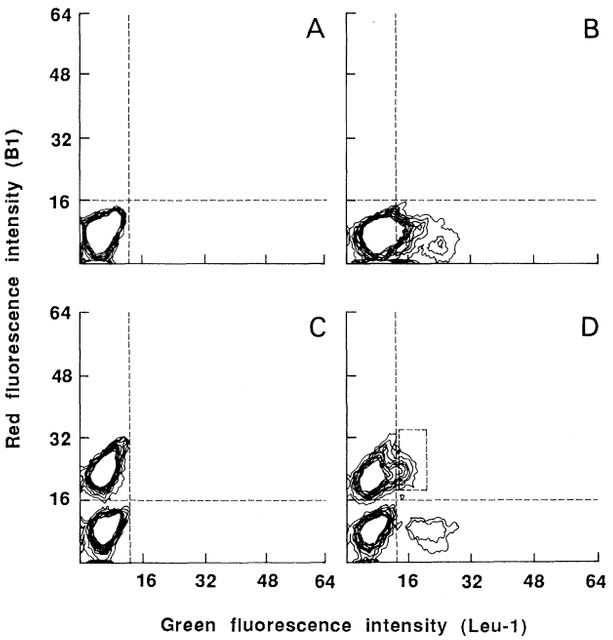

Fig. 1. Identification and quantitation of human Leu-1^+ B lymphocytes in peripheral blood from a healthy subject: two-color FACS analysis. Approximately 10^6 B cells (*12*) were incubated for 45 minutes in 0.1 ml of ice-chilled Hanks balanced salt solution without Ca^{2+}, Mg^{2+}, or phenol red and with 1% bovine serum albumin (BSA-HBSS) containing (**A**) biotinylated IgG2a mAb with irrelevant specificity (1 μg); (**B**) biotinylated IgG2a mAb to Leu-1 (0.25 μg, Becton and Dickinson; Mountain View, California); (**C**) phycoerythrin-conjugated mouse mAb to B1 (1 μg of PE-conjugated mAb to B1, Coulter, Hialeah, Florida); (**D**) both PE-conjugated mAb to B1 and biotinylated mAb to Leu-1. All cell samples then were washed, suspended in 0.1 ml of BSA-HBSS containing FITC-avidin (1.5 μg; Sigma, St. Louis, Missouri), and incubated for another 45 minutes in ice. After further washing, the cells were submitted to FACS analysis (FACS 440 equipped with an argon laser, Becton and Dickinson). Fluorescein (green) and phycoerythrin (red) emission lights were collected with appropriate filters at 525 and 575 nm, respectively. Contourgrams are the graphic representation of three-dimensional surfaces in which coordinates of the green and red fluorescence intensities of individual cells define the location of cells on a 64 by 64 grid, and the frequency of cells at each location defines the elevation of that location. Contourgrams were computer-generated to represent equal step changes in elevation. Cells showing both red fluorescence ($B1^+$) and green fluorescence (Leu-1^+) within the limits of the rectangle identified by the dotted lines in the upper right quadrant of (D) constituted 14.0% of total B cells and were considered Leu-1^+ B lymphocytes. The cells in the lower right quadrant of (D) showing only green fluorescence (Leu-1^+) constituted residual T cells.

Leu-1 (namely, B36.1) and B cells (OKB7 and B2) (*13*).

To delineate the functional characteristics of the Leu-1^+ B lymphocytes, we used FACS to separate Leu-1^+ B cells from Leu-1^- B cells. Peripheral blood B lymphocytes were simultaneously treated with PE-conjugated mAb to B1 and biotinylated mAb to Leu-1, incubated with FITC-avidin, and then applied to the FACS. Red fluorescent B cells were "gated," and Leu-1^+ and Leu-1^- subsets were identified and sorted (Fig. 2A). After the sorting procedure was completed, B cells from the putative Leu-1^- and Leu-1^+ fractions were reapplied to the FACS to verify the degree of separation previously achieved. All negatively (Leu-1^-) sorted cells (Fig. 2B) and most of the positively (Leu-1^+) sorted cells (Fig. 2C) fell within the coordinates established for the sorting procedure.

To study the capacity of the sorted cells to make antibodies, we transformed Leu-1^+ and Leu-1^- B lymphocytes into Ig-secreting cells by infection with EBV (*11, 14*). After infection, the cells were distributed at various doses in 96-well, U-bottom plates containing 10^5 irradiated (2500 rads) human peripheral blood mononuclear cells as feeder layers (*11, 15*). Unfractionated B cells (B1-positive cells passed through the FACS) were similarly processed. After 4 weeks of incubation, culture fluids were harvested and investigated for their Ig content with a battery of enzyme-linked immunosorbent assay (ELISA) plates suitable for the titration of total Ig of various isotypes. Table 1 shows that equal numbers of Leu-1^+, Leu-1^-, or unfractionated B lymphocytes from the same donor secreted roughly comparable amounts of total IgM, IgG, IgA, and IgD after infection with EBV. Moreover, the frequency of B lymphocytes secreting Ig of various isotypes after infection with EBV was approximately the same among Leu-1^+, Leu-1^-, and unfractionated B cells (Table 1). The binding activity of Ig produced by EBV-infected Leu-1^+, Leu-1^-, and unfractionated B lymphocytes was then investigat-

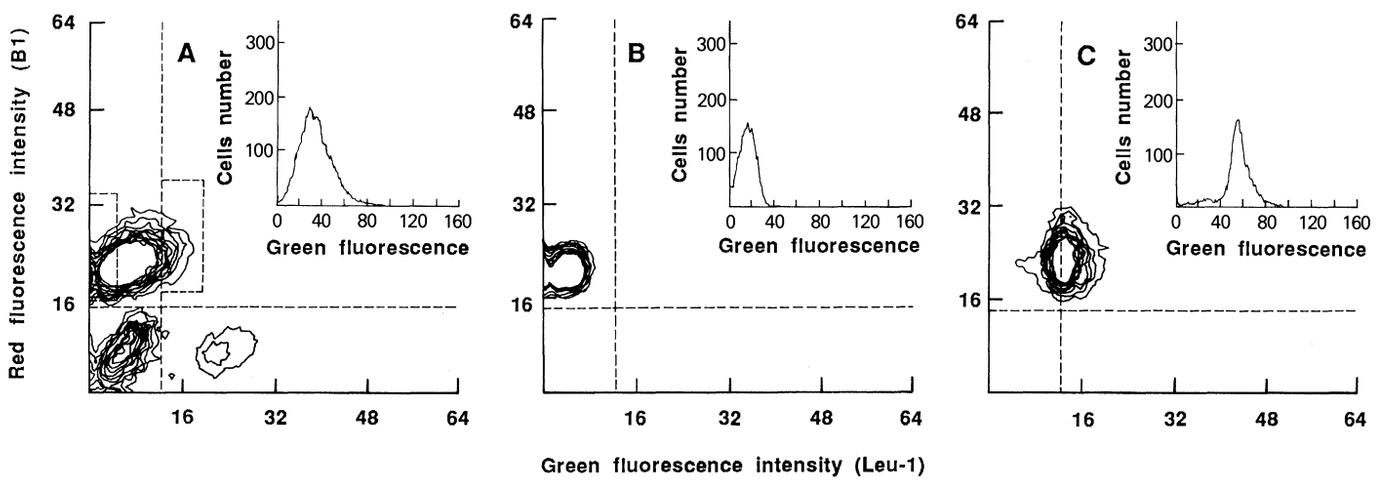

Fig. 2. Sorting of Leu-1^+ cells from peripheral blood B lymphocytes of a healthy subject. B lymphocytes (5×10^7) were simultaneously reacted with PE-conjugated mAb to B1 (50 μg) and biotinylated mAb to Leu-1 (12.5 μg), washed, and then reacted with FITC-avidin (50 μg). After further washing, cells were applied to the FACS for analysis. Leu-1^+ B lymphocytes were identified as cells showing both red fluorescence ($B1^+$) and green fluorescence (Leu-1^+). (**A**) Cells included within the left dotted (20% of total B cells) and right dotted (10% of total B cells) rectangles were considered Leu-1^- and Leu-1^+ B lymphocytes, respectively. These two cell fractions were then sorted, collected, and reapplied to the FACS to evaluate the degree of separation. (**B** and **C**) These contourgrams show that all the negatively sorted cells (Leu-1^-) and most of the positively sorted cells (Leu-1^+) fell back within the coordinates respectively established for the sorting procedure. Insets represent the green fluorescence profiles of B cells analyzed in contourgrams A, B, and C. Contourgrams and profiles show that the sorted Leu-1^+ and Leu-1^- B cell fractions overlap only marginally.

ed. ELISA plates coated with purified Fc fragment of human IgG, purified ssDNA, or purified tetanus toxoid (TT) were used to measure antibody activity in the culture fluids (*16*). Virtually all lymphocytes producing antibodies (IgM) to the Fc fragment of human IgG and to ssDNA segregated with the Leu-1$^+$ B cells (Fig. 3). Similarly, production of IgG antibodies to ssDNA was predominantly associated with Leu-1$^+$ B cells. In contrast, when the binding activity to TT, a common exogenous molecule, was investigated, it was found that all IgG molecules to TT were made by the Leu-1$^-$ B cells, whereas the IgM molecules to TT were made predominantly by the Leu-1$^+$ B lymphocytes. Similar data were obtained in separate experiments from five other healthy subjects.

The present studies show (i) that Leu-1$^+$ B lymphocytes can easily be detected in relative large numbers in the peripheral blood of healthy subjects by double-fluorescence flow cytometry; (ii) that these Leu-1$^+$ B lymphocytes can be separated as a discrete cell subset from most B cells (Leu-1$^-$) by FACS and driven to secrete Ig molecules of the four major isotypes (IgM, IgG, IgA, and IgD) by infection with EBV; (iii) that virtually all lymphocytes capable of producing antibodies to the Fc fragment of human IgG and ssDNA segregate with the Leu-1$^+$ B cell subset; and (iv) that lymphocytes producing IgG antibodies to at least one exogenous molecule, TT, segregate with the Leu-1$^-$ B cell population.

The definition of the window chosen to sort Leu-1$^+$ B lymphocytes from peripheral blood B cells was necessarily arbitrary. However, the number of Leu-1$^+$ B cells detected (about 17% of total B cells) probably represents a more realistic measure than the 3% previously reported (*7*) by simple fluorescence microscopy, a much less sensitive procedure than FACS for appreciating "dim" fluorescent cells. In our experiments, the verification that B cells originally defined as Leu-1$^+$ were indeed Leu-1$^+$ came from the results of studies in which B cells sorted as Leu-1$^+$ did have the same coordinates on reanalysis (Fig. 2). Our experiments also established that human Leu-1$^+$ B lymphocytes could be infected by EBV as efficiently as Leu-1$^-$ B lymphocytes (*17*). Moreover, the amount of IgM, IgG, IgA, or IgD secreted by EBV-infected Leu-1$^+$ B cells did not significantly differ from that of Leu-1$^-$ B cells, or for that matter unfractionated B lymphocytes (Table 1).

In terms of functional properties, the Leu-1$^+$ B cell fraction from normal healthy subjects contained most of the lymphocytes capable of producing antibodies to ssDNA and the Fc fragment of IgG. In contrast, the Leu-1$^-$ B cell fraction contained few or no lymphocytes capable of making these autoantibodies. In other experiments, lymphocytes that made IgM antibodies to other autoantigens, insulin and thyroglobulin, also segregated primarily with the Leu-1$^+$ B cell subset. However, IgM antibodies to at least one "exogenous" antigen, TT, were also produced by Leu-1$^+$ B cells. Similar results were recently obtained with another exogenous antigen, β-galactosidase from

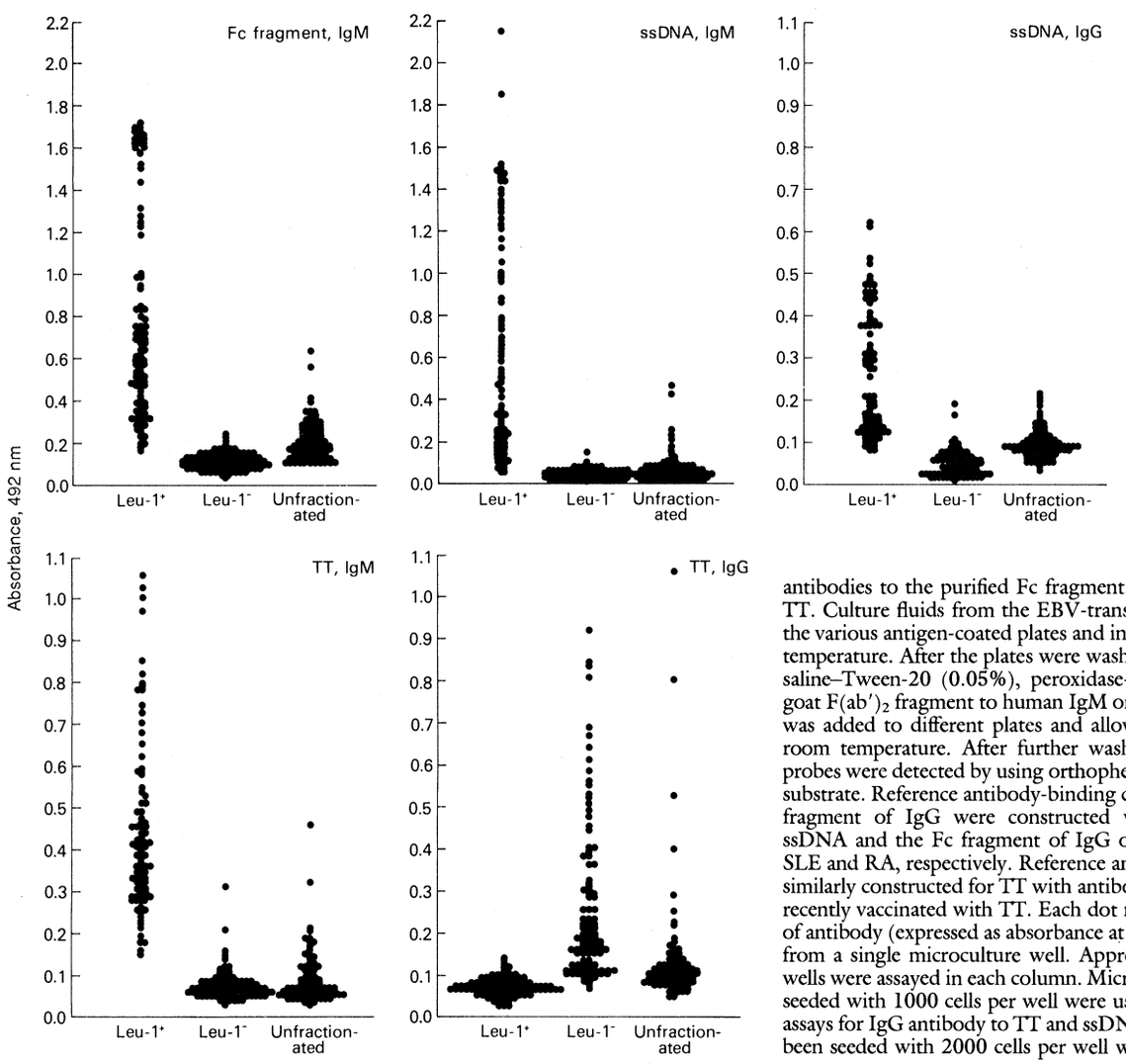

Fig. 3. Antibodies produced by human Leu-1$^+$, Leu-1$^-$, and unfractionated B lymphocytes infected with EBV. Microculture plates were seeded with EBV-infected cells at various doses in the presence of irradiated feeder layers (Table 1). After 4 weeks of culture, fluids were tested for antibody activity. Enzyme-linked immunosorbent assays were used for the titration of antibodies to the purified Fc fragment of human IgG, ssDNA, or TT. Culture fluids from the EBV-transformed cells were added to the various antigen-coated plates and incubated for 2 hours at room temperature. After the plates were washed with phosphate-buffered saline–Tween-20 (0.05%), peroxidase-conjugated affinity-purified goat F(ab')$_2$ fragment to human IgM or IgG (Cooper Biochemical) was added to different plates and allowed to react for 2 hours at room temperature. After further washing, bound enzyme-linked probes were detected by using orthophenylenediamine and H_2O_2 as substrate. Reference antibody-binding curves for ssDNA and the Fc fragment of IgG were constructed with human antibodies to ssDNA and the Fc fragment of IgG obtained from patients with SLE and RA, respectively. Reference antibody-binding curves were similarly constructed for TT with antibodies obtained from subjects recently vaccinated with TT. Each dot represents the concentration of antibody (expressed as absorbance at 492 nm) in the culture fluid from a single microculture well. Approximately 100 microculture wells were assayed in each column. Microculture wells that had been seeded with 1000 cells per well were used, the exception being the assays for IgG antibody to TT and ssDNA, for which plates that had been seeded with 2000 cells per well were used.

Table 1. Production of IgM, IgG, IgA, or IgD by EBV-infected Leu-1$^+$, Leu-1$^-$, and unfractionated B lymphocytes. Leu-1$^+$ and Leu-1$^-$ B lymphocytes from one donor were selected by FACS. Unfractionated B lymphocytes comprised the whole B cell fraction passed through the FACS. Cells were infected with EBV and cultured at various doses in 96-well, U-bottom plates in the presence of 10^5 irradiated peripheral blood mononuclear cells as feeders. After 4 weeks, culture fluids were assayed for Ig content by ELISA plates coated with goat F(ab')$_2$ fragment to human Ig (IgM + IgG + IgA) or to human IgD by a procedure similar to that described in the legend to Fig. 3. Bound immunoglobulins of various isotypes were detected by peroxidase-conjugated affinity-purified goat F(ab')$_2$ probes to human IgM, IgG, IgA, or IgD (Cooper Biochemical). Reference curves were constructed with purified human IgM, IgG, IgA, or IgD and used to estimate the Ig content (expressed in nanogram equivalents) of culture fluids. The amount of Ig produced is given in nanogram equivalents of Ig produced by 500 cells over a period of 4 weeks and shown as the mean ± SD from 176 microculture wells. All wells were positive for cell transformation and Ig production. The fraction of B lymphocytes producing Ig of a given class was calculated by limiting dilution methodology and analysis according to Poisson distribution (*15*). Cells were cultured in amounts of 2000, 1000, 500, 100, 50, 20, 10, 5, and 2.5 per well. Forty-eight microcultures were used for each cell dose.

Ig class	Leu-1$^+$		Leu-1$^-$		Unfractionated	
	Amount of Ig produced	Fraction producing Ig	Amount of Ig produced	Fraction producing Ig	Amount of Ig produced	Fraction producing Ig
IgM	7000 ± 692	1/3	7600 ± 754	1/3	8250 ± 479	1/3
IgG	200 ± 36	1/70	310 ± 44	1/55	395 ± 40	1/60
IgA	71 ± 11	1/65	77 ± 32	1/75	105 ± 7	1/70
IgD	62 ± 13	1/65	75 ± 19	1/75	75 ± 10	1/70

Escherichia coli. These findings, although still limited in scope, suggest that the label "autoantibody-producing B lymphocytes" that one might be tempted to attach to Leu-1$^+$, and perhaps Ly-1$^+$ B cells, may not be appropriate. In fact, preliminary data from our laboratory suggest that the Ig produced by Leu-1$^+$ B cells from normal subjects constitute the class of antibodies that have been variously described as multiple organ–reactive or polyreactive (*11, 15, 18, 19*). It has been suggested that the V_H genes used by cells that make these antibodies are in the unmutated (germline) configuration (*19–22*). The fact that the Leu-1$^+$ subset of B cells is the primary source of antibodies to ssDNA and the Fc fragment makes it possible that only a selected number of V_H gene families are involved in the production of these autoantibodies. This also would be compatible with the finding that polyreactive autoantibody molecules show a high incidence of idiotypic cross-reactivity in humans and mice (*19, 20, 22–24*).

In contrast to their IgM-producing counterparts that segregated with the Leu-1$^+$ B cells, lymphocytes producing IgG to TT segregated with the Leu-1$^-$ B cells. This raises the possibility that lymphocyte clones that had previously gone through genuine antigen-driven amplification and mutation by an exogenous molecule, as is likely the case with B lymphocytes producing IgG to TT, belong to the "conventional" compartment (Leu-1$^-$) of the B cell repertoire. Further studies are needed, however, to determine whether the Leu-1$^-$ B cells producing IgG to TT originally stemmed from TT-binding Leu-1$^-$ B cells or possibly from Leu-1$^+$ B cells that differentiated into Leu-1$^-$ B cells. There is some evidence that expression of Leu-1 may be associated with an early stage of B cell differentiation. Indeed, "dim" Leu-1$^+$ B lymphocytes are present in large numbers in fetal spleen and lymph nodes (*8, 9*) and constitute the majority of graft-derived B lymphocytes that repopulate the host after bone marrow transplantation (*10*). In this context, chronic B-lymphocytic leukemia cells, which consistently express the Leu-1 marker, may represent the neoplastic counterpart of the early stage of B cell differentiation (*6*).

What is the relevance of our findings to autoimmunity? Antibodies binding to ssDNA and the Fc fragment of human IgG are characteristic of at least two important human diseases, SLE and RA. Several studies indicated that healthy individuals have circulating B lymphocytes capable of producing antibodies to ssDNA, the Fc fragment of IgG, and other self-antigens (*11, 15, 18–20, 23*). We showed that these lymphocytes constitute a discrete B cell subset that can be segregated by a distinct surface marker (Leu-1). In healthy humans these Leu-1$^+$ B cells are most likely present in a resting state, associated with no or a few circulating antibodies. Activation of these cells in vivo could be associated with the accumulation of circulating autoantibodies (*7*). The demonstration that a discrete subset of B cells (Leu-1$^+$) makes autoantibodies raises the possibility that, in diseases such as SLE, RA, or Sjögren's syndrome, the amplification of autoantibody-producing B cell clones may not necessarily be an antigen-driven process but could be due to a variety of factors including viruses, oncogenes, or growth factors. The findings that Leu-1$^+$ cells are present in significant numbers in the normal human B cell repertoire, that they can be segregated by FACS from the Leu-1$^-$ B cells, and that both Leu-1$^+$ and Leu-1$^-$ B cells can be transformed by EBV to make antibodies with discrete reactivities should make it possible to evaluate the roles of these cells in autoimmune disorders.

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12. Mononuclear cells were purified from human peripheral blood of healthy donors (Leukophoresis Research Program, NIH Blood Bank, NIH, Bethesda, MD) or from spleens of healthy subjects, who died in accidents, by centrifugation through lymphocyte-separating medium (Bionetics, Rockville, MD). Some of the blood donors had been previously vaccinated with tetanus toxoid. Monocytes were removed from mononuclear cells by incubation with carbonyl iron particles and the iron-loaded monocytes were subsequently removed by a magnet. The mononuclear cells were depleted of T cells by incubation (in ice) with AET (2-aminoethyl-isothioronium bromide hydrobromide)-treated sheep red blood cells (SRBC). The non-SRBC rosette-forming cells consisted of at least 50% B cells, some residual monocytes and T cells, and variable numbers of lymphocytes with the NK phenotype, as assessed by mouse monoclonal antibodies OKB7, B77.1, OKT3, and B73.1, respectively. This non-SRBC rosetting, enriched B lymphocyte fraction is referred to here as B cells.
13. B36.1 is an IgG2b mAb to Leu-1 (CD5). OKB7 and B2 are two different mAbs to the C3d receptor (CD21) present on B lymphocytes. OKB7 is an IgG2a (Ortho; Raritan, NJ) and B2 is an IgM (Coulter).
14. EBV used to infect B lymphocytes was obtained from a culture fluid of B95-8 marmoset lymphoma cells. This virus preparation had a titer of 5×10^5 transforming units per milliliter, one transforming unit being the minimal amount of virus-producing transformation of 10^4 purified human B cells.
15. M. Nakamura, T. S. Davies, G. Inghirami, A. L. Notkins, P. Casali, in preparation.
16. Calf thymus ssDNA was prepared by boiling (10 minutes) highly polymerized DNA (Sigma, St. Louis, MO) followed by chilling in ice; purified Fc fragment of human IgG was obtained from Cooper Biochemical (Malvern, PA); and semipurified TT was obtained from the Commonwealth of Massachusetts (Department of Health, Boston, MA) and fractionated to homogeneity by gel filtration on a Sephadex (Pharmacia) G-150 column. Polystyrene plates (Immulon II, Dynatech, McLean, VA) were coated with Fc fragment (5 μg/ml), ssDNA (5 μg/ml), or TT (1 μg/ml) in 0.1*M* carbonate buffer, *p*H 9.5, during a 24-hour incubation period at 4°C. Plates were stored at −20°C until used.
17. Limiting dilution methodology and analysis according to Poisson distribution showed that the immortalization rates of EBV-transformed Leu-1$^+$, Leu-1$^-$, and unfractionated B cells were 1/56, 1/48, and 1/50, respectively.
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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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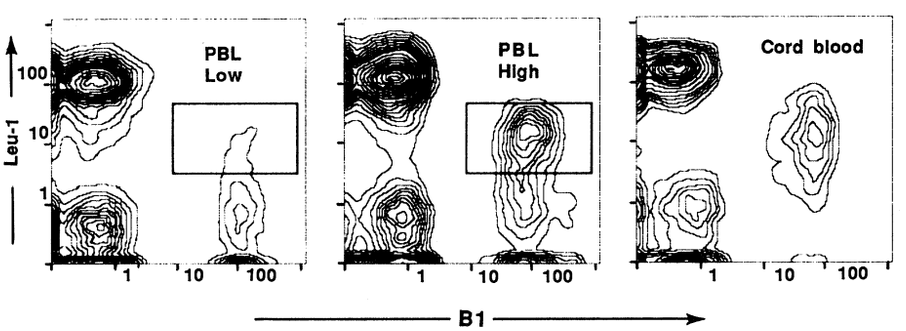

Fig. 1. Peripheral blood of adults contains both high and low phenotypes of Leu-1$^+$ B cells, whereas B cells from cord blood are largely Leu-1$^+$. Ficoll-separated PBLs or cord blood cells were stained with fluorescein-conjugated B1 (Coulter) together with biotin-conjugated antibody to Leu-1 (Becton 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.