B-LYMPHOCYTE HETEROGENEITY: ONTOGENETIC DEVELOPMENT AND ORGAN DISTRIBUTION OF B-LYMPHOCYTE POPULATIONS DEFINED BY THEIR DENSITY OF SURFACE IMMUNOGLOBULIN*

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The density of immunoglobulin (Ig) on the surface of mouse bone marrow-derived (B) lymphocytes has recently been studied by two groups of investigators (1–3). Both groups attributed developmental significance to differences in the density of Ig on the surface of these cells, but Osmond and Nossal concluded that a substantially greater heterogeneity in the amount of Ig per B cell existed in the immature population vs. the mature population, whereas Sidman and Unanue indicated that immature cells have, on the average, more Ig than mature cells. The techniques employed in these studies are very tedious and are, therefore, poorly suited to a detailed analysis of the distribution of the density of surface Ig on different populations of B lymphocytes. However, the density of surface Ig on individual B lymphocytes derived from different sources is critical to the understanding of antigen-B-lymphocyte interaction and activation. The introduction of the technique of rapid flow microfluorometry (RFMF)[1] and electronic cell sorting makes possible a direct approach to the study of surface Ig density on B lymphocytes and to an evaluation of its developmental and functional significance.

Using this technology, we report here the identification among splenic B lymphocytes of a large subpopulation of cells possessing a relatively low-to-intermediate density of surface Ig when a fluorescein-conjugated (Fl) anti-mouse Ig or anti-κ is used to detect Ig-bearing cells. Such B lymphocytes, as

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[1] *Abbreviations used in this paper:* FACS, Fluorescence Activated Cell Sorter; FCS, fetal calf serum; FITC, fluorescein; Fl, fluorescein-conjugated; Mls, minor lymphocyte stimulating; *nu/nu*, congenitally athymic; RFMF, rapid flow microfluorometry.

detected by a peak in the fluorescence profile, are found in the spleen and lymph nodes of adult mice. A similar peak is not detected when neonatal spleen or adult bone marrow cells are examined. The development of this population of B lymphocytes occurs during the first 4 wk of life and appears to be independent of the influence of thymus-derived (T) lymphocytes or of antigenic stimulation, since the fluorescence profiles of spleen cells of adult congenitally athymic (*nu/nu*) mice and of mice raised under germfree conditions are indistinguishable from those of normal adult animals. Parallel studies using Fl anti-μ to identify surface IgM failed to demonstrate the distinct B-lymphocyte subpopulation with low-to-intermediate fluorescence intensity among adult spleen cells. Moreover, the fluorescence profiles of Fl anti-μ-labeled adult bone marrow and adult spleen were similar to each other.

Materials and Methods

Animals. DBA/2N, C57BL/6N, and BALB/cAnN (germfree or conventionally reared) mice were obtained from the Animal Production Section, Division of Research Services of the National Institutes of Health, Bethesda, Md. CBA/J and C3H/HeJ mice were purchased from The Jackson Laboratory, Bar Harbor, Maine. BALB/c *nu/nu* and +/*nu* littermates which were in the seventh backcross generation were obtained from the Charles River Breeding Laboratories, Inc., Wilmington, Mass. Mice were from 8 to 12 wk of age at the time of study unless otherwise noted. In all instances, lymphoid organs from individual mice were studied, except when newborn to 3-wk-old mice were analyzed, when the lymphoid organs of four to five individual mice were pooled.

Cell Suspensions. Spleens; Peyer's patches; popliteal, inguinal, and cervical lymph nodes; and tibiae and fibulae were obtained from mice killed by cervical dislocation. Suspensions of spleen, Peyer's patches, and lymph node cells were prepared by teasing of these organs with forceps into RPMI 1640 (Grand Island Biological Co., Grand Island, N. Y.) while bone marrow cells were flushed from the shaft of the tibia or fibula using a 25 gauge needle and syringe. Cell aggregates were dispersed by passage through a 26 gauge needle. Spleen and bone marrow cells were centrifuged at 100 *g* for 10 min, the supernatant fluid removed, and the pellet treated with 5 ml of 0.83% ammonium chloride at 4°C for 5 min to lyse red blood cells. Nucleated cells obtained by this method were washed through fetal calf serum (FCS) (Grand Island Biological Co.) to remove red blood cell ghosts.

Anti-Immunoglobulin Reagents. A polyspecific goat anti-mouse 7S globulin and an anti-mouse-μ were obtained from Meloy Laboratories Inc., Springfield, Va. (Lot nos. 40691 and 40643, respectively). These antisera were digested with 2% (wt/wt) pepsin (Mann Research Labs, New York) in acetate buffer (pH 4.0) for 18 h, and $F(ab')_2$ fragments were further purified on a Sephadex G-200 column (100 × 1 cm), dialyzed against a bicarbonate buffer (pH 9.3), and concentrated to 4 mg/ml. $F(ab')_2$ fragments were conjugated with fluorescein isothiocyanate (BBL, Division of Becton, Dickinson & Co., Cockeysville, Md.) by overnight dialysis of 10 ml of protein (10 mg) in a bicarbonate buffer (pH 9) against 1 mg of FITC in 1 ml of the buffer enclosed within a dialysis bag. Fl $F(ab')_2$ antisera with a molar ratio of protein to fluorescein of approximately 2:1 were isolated from proteins with a higher or lower protein-to-fluorescein ratio using DEAE-cellulose column chromatography. The Fl $F(ab')_2$ anti-Ig and anti-μ will be referred to as the Fl anti-Ig and Fl anti-μ, respectively. In addition to these preparations, a series of fluoresceinated affinity column-purified antibodies was also used in these studies. These include goat anti-mouse γ_1-, γ_2-, α-, and μ-antibodies (4) and rabbit anti-mouse-κ antibody (a gift from Dr. R. Mage, NIH, Bethesda, Md.).

In order to further analyze the specificity of the Fl anti-Ig, 0.8 mg (0.2 ml) of this reagent was passed through a 1 ml column containing 200 μg each of $IgG_1(\kappa)$, $IgG_{2a}(\kappa)$, and $IgG_{2b}(\kappa)$ myeloma proteins bound to Sepharose. The column was eluted with 5 cm^3 of borate buffer and the effluent was dialyzed against saline and concentrated to the original protein concentration. The molar ratio of protein to fluorescein of this material was similar to that of the original. As noted in the Results section, absorption of the anti-Ig with these three mouse myeloma proteins abolished the ability of this antisera to stain mouse spleen cells.

Lymphocyte Staining. Nucleated cells obtained from lymph node or ammonium chloride-treated bone marrow or spleen cell suspensions were washed twice with RPMI 1640 and adjusted to 50×10^6 cells/ml with RPMI 1640 containing 5% FCS and 1 mg/ml of sodium azide. Preliminary studies indicated that 10×10^6 cells in 200 μl would be optimally labeled for use with the Fluorescence Activated Cell Sorter (FACS) (Becton, Dickinson Electronics Laboratory, Mountain View, Calif.) with 20 μg of Fl anti-Ig; 10 μg of Fl anti-μ; or 2.5 μg of the affinity column purified anti-μ, anti-κ, anti-γ_1, anti-γ_2, and anti-α. The undigested Fl antibodies (anti-κ, anti-μ, anti-γ_1, anti-γ_2, and anti-α) were ultracentrifuged for 40 min at 100,000 *g* before their use. Cell suspensions were incubated with Fl antibodies at 4°C for 30 min, washed three times in cold RPMI 1640 (5% FCS, 1 mg/ml sodium azide), and resuspended in the same media to 10×10^6 cells/ml.

Flow Microfluorometry. A detailed explanation of the principle of RFMF has been presented by Loken and Herzenberg (5). The frequency and fluorescence profile of cells stained with Fl antisera was determined after examining at least 100,000 viable cells. Cellular volume was determined by a Coulter Counter (Model B; Coulter Electronics Inc., Hialeah, Fla.) which was made available by Dr. C. Herman, NIH, Bethesda, Md.

Results

Fluorescence Profile of Murine Lymphocytes Stained with Fl Anti-Ig. The fluorescence profiles of unstained and Fl anti-Ig-stained CBA/J thymus and spleen cells are shown in Figs. 1*a* and *b*. The horizontal axis of these and all subsequent fluorescence profiles displays 1,000 linear channels of increasing fluorescence intensity toward the right, while the vertical axis displays the number of individual cells within each channel. Less than 0.2% of CBA/J thymus cells were stained by the Fl anti-Ig, indicating that this reagent was not detecting species-specific surface antigens found on mouse thymocytes. By contrast, when CBA/J splenic lymphocytes stained with Fl anti-Ig were studied by RFMF, 54.5% of the cells were found to be fluorescent. The fluorescence profile of these Fl anti-Ig-stained splenic B lymphocytes was characterized by a large peak of cells which has a low-to-intermediate degree of fluorescence intensity. Similar fluorescence profiles were obtained when DBA/2N (Fig. 2*a*), C57BL/6, BALB/c, and C3H/HeJ splenic lymphocytes were stained with Fl anti-Ig. Thus, the existence of a large number of cells with a low-to-intermediate degree of fluorescence intensity as a dominant feature of the fluorescence profile of adult splenic B lymphocytes appears to be a general phenomenon, as it is found in spleen cells from five different inbred mouse strains.

In order to further characterize the Fl anti-Ig, we stained C57BL/6 spleen cells with this reagent and compared the profile to that of C57BL/6 stained cells stained with Fl anti-κ. The fluorescence profiles of these cells after labeling with either Fl anti-Ig or Fl anti-κ were virtually identical and the frequency of Ig-positive cells were 44.7 and 46.5%, respectively. Moreover, after absorption of the Fl anti-Ig on a Sepharose column containing IgG_1, IgG_{2a}, and IgG_{2b} mouse myeloma proteins, as described in the Materials and Methods section, its capacity to label splenic B lymphocytes was abolished.

The large number of splenic B lymphocytes displaying a low-to-intermediate degree of fluorescence after staining with Fl anti-Ig could be accounted for by (*a*) the presence of cells of a uniform volume which contained a major population of cells with a low-to-intermediate density of surface Ig, (*b*) the presence of cells with a uniform density of surface Ig which contains a large population of cells with a low-to-intermediate cellular volume, or (*c*) populations of cells varying in

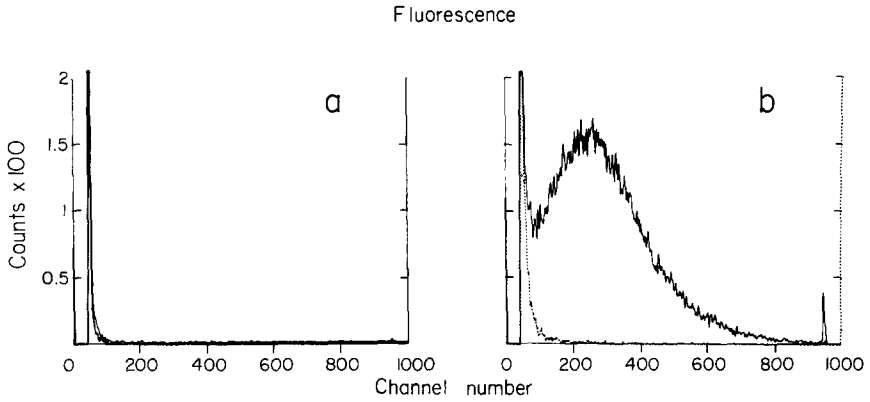

FIG. 1. Fluorescence profiles of (*a*) CBA/J thymus cells, unlabeled (---) or labeled with Fl anti-Ig (—); (*b*) CBA/J spleen cells, unlabeled (---) or labeled with Fl anti-Ig (—).

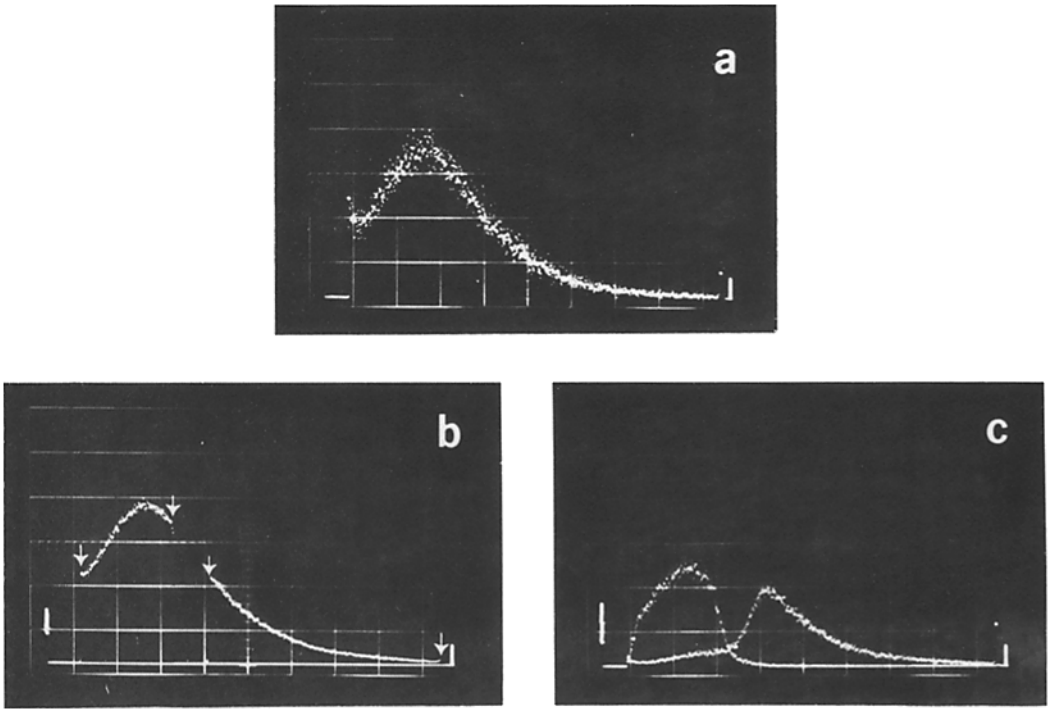

FIG. 2. Fluorescence profiles of (*a*) DBA/2 spleen cells labeled with Fl anti-Ig, (*b*) DBA/2 spleen cells labeled with Fl anti-Ig and separated according to their relative fluorescence intensity as noted by the arrows, and (*c*) DBA/2 spleen cells labeled with Fl anti-Ig after separation. Fig. 3 *c* is a composite picture of individual analytical runs of cells that were separated as shown in Fig. 3 *b*.

their volume and density of surface Ig. In order to distinguish between these possibilities, spleen cells from DBA/2N mice were stained with Fl anti-Ig (Fig. 2*a*) and cells with low fluorescence intensity were separated from those with high fluorescence intensity using the FACS (Fig. 2*b*). When these cells were re-

examined with the FACS, the separation of low from high intensity fluorescence cells was confirmed (Fig. 2*c*). The average cellular volumes of these two populations were 119 and 128 μm^3 for the "bright" and low-to-intermediate fluorescence intensity B lymphocytes, respectively. This indicates that the distinct fluorescence intensities of these two populations does not reflect differences in their cellular volumes, but is secondary to differences in the density of Ig on their surfaces.

Fluorescence Profiles of Bone Marrow, Lymph Node, or Peyer's Patch Lymphocytes Stained with Fl anti-κ. The fluorescence profiles of bone marrow, lymph node, and Peyer's patch lymphocytes stained with Fl anti-κ are shown in Figs. 3*a–c*, respectively. In order to facilitate the comparison of these profiles with those obtained with splenic lymphocytes, the scale (counts × 100) of the bone marrow, lymph node, and Peyer's patch plots was changed so that the areas under the Ig-positive portion of the curves (channels 80–1,000) would equal that of the splenic profile and the two profiles were plotted together. The frequency of Ig-positive cells in the bone marrow, lymph nodes, and Peyer's patches was 18.9, 18.4, and 55.2%, respectively. B lymphocytes derived from CBA/J lymph nodes (Fig. 3*b*) had a fluorescence profile which was very similar to the profile of splenic B lymphocytes. The profile obtained with Peyer's patch cells (Fig. 3*c*) demonstrates that a large fraction of the cells have a very low fluorescence intensity and are poorly separated from background. No fluorescence peak was seen when bone marrow cells (Fig. 3*a*) were labeled with Fl anti-κ. Moreover, there were substantial numbers of very intensely stained B lymphocytes in bone marrow, and the relative frequency of bright cells in this organ was greater than in spleen.

Fluorescence Profile of Neonatal Spleen Cells Stained with Fl Anti-Ig. The absence of the fluorescence peak among bone marrow cells stained with Fl anti-Ig suggested that the cells within the low-to-intermediate fluorescence peak seen in the spleens and lymph nodes of adult mice represented a mature subpopulation of B lymphocytes. This was further studied by examining the fluorescence profile of spleen cells derived from 3-, 10-, 17-, and 30-day-old and adult BALB/c mice (Figs. 4*a–e*). In the first 17 days of life, the relative frequency of cells with a very low-to-low (channels 80–200) and low-to-intermediate (channels 200–400) fluorescence intensity among the Ig-positive cells (channels 80–1,000) increases so that at 17 days they represent 44.2 and 42.1% of the Ig-positive cells, respectively (Table I). These changes are associated with a marked increase in the overall frequency of Ig-positive cells (29.2 to 54.0%). After 17 days, the frequency of cells with a very low-to-low fluorescence intensity begins to decrease and the continued decline in this population associated with an increase in the relative frequency of cells with a low-to-intermediate fluorescence intensity results in the characteristic Fl anti-Ig-labeled adult profile (Fig. 4 *e*).

Fluorescence Profiles of nu/nu and Germfree BALB/c Splenic B Lymphocytes Stained with Fl Anti-Ig. After labeling with Fl anti-Ig, the fluorescence profile and frequency of Ig-positive cells among spleen cells derived from conventionally or germfree-reared adult BALB/c mice were essentially identical (data not shown). The fluorescence patterns of *nu/nu* and *+/nu* spleen cells labeled with

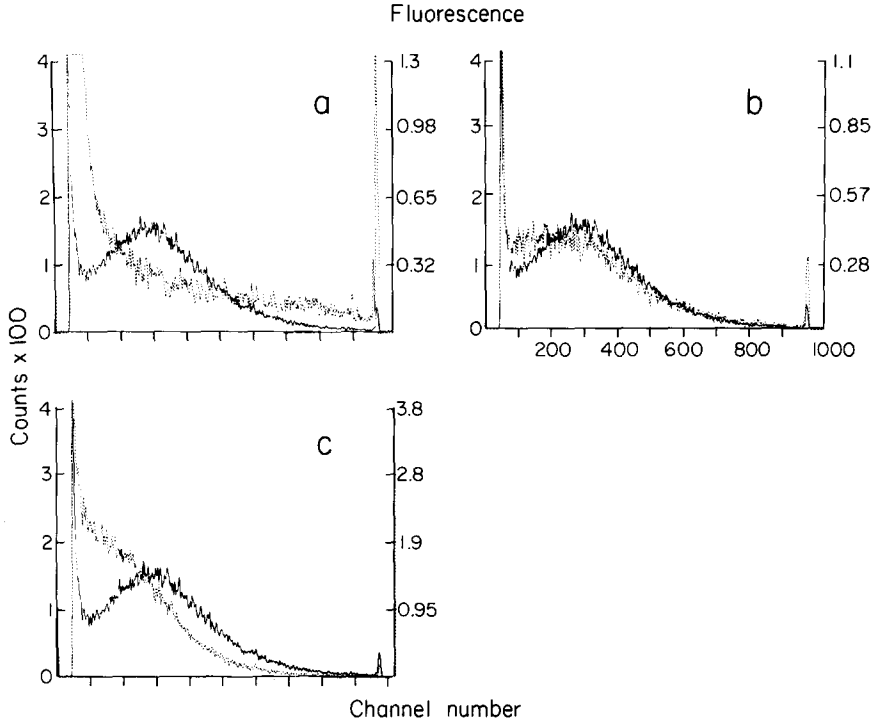

FIG. 3. Fluorescence profiles of (*a–c*) CBA/J spleen cells labeled with Fl anti-Ig (—) compared to (*a*) CBA/J bone marrow cells (---), (*b*) CBA/J lymph node cells (---), and (*c*) CBA/J Peyer's patch cells (---), labeled with Fl anti-Ig. The profiles of the bone marrow, lymph node, and Peyer's patch cells were plotted so that the areas under the Ig-positive portion of their fluorescence profiles were equal to that of the spleen cell profile.

Fl anti-Ig were also similar to each other and to that of normal adult cells (Fig. 5). However, the frequency of Ig-positive cells in BALB/c *nu/nu* spleen cell suspensions was considerably greater (87.1%) than seen with the *+/nu* spleens (50.6%).

Fluorescence Profiles of Lymphocytes Stained with Fl Anti-Heavy Chain Antibodies. The frequency of fluorescent cells after labeling adult CBA/J spleen cells with Fl anti-γ_1, Fl anti-γ_2, Fl anti-α, and Fl anti-μ was <1, 3.8, <1, and 54.0%, respectively. Although the Fl anti-μ stained an equivalent percentage of spleen cells when compared to Fl anti-κ (54 vs. 54.1%), a fluorescence peak was not seen after labeling with Fl anti-μ (Fig. 6*a*).

When B lymphocytes from CBA/J adult bone marrow, lymph node, and Peyer's patches were stained with Fl anti-μ, the profiles obtained were generally similar to each other and to those observed with adult spleen cells (Figs. 6*b–d*). In no case, was a low-to-intermediate intensity fluorescence peak observed. However, the relative frequency of cells with a very low-to-intermediate density of surface IgM among the Ig-positive cells was greater in lymph node and Peyer's patches (86.0 and 89.4%, respectively) than spleen and bone marrow (74.2 and 71.7%, respectively). The frequency of Ig-positive lymphocytes, detected after labeling with Fl anti-μ, was 19.8, 16.6, and 33.8% when cells derived

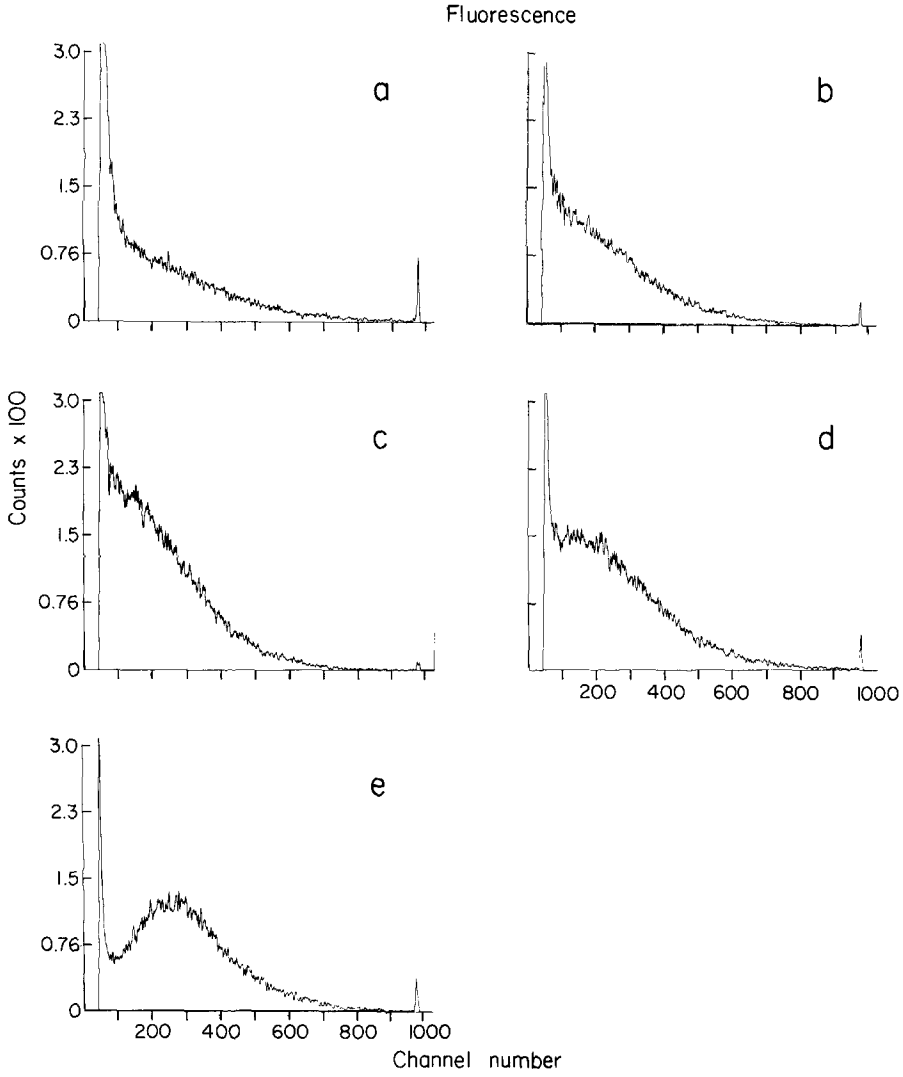

FIG. 4. Fluorescence profiles of BALB/c spleen cells labeled with Fl anti-Ig from (*a*) 3-day-old, (*b*) 10-day-old, (*c*) 17-day-old, (*d*) 30-day-old, and (*e*) adult mice.

from CBA/J, bone marrow, lymph nodes, or Peyer's patches were examined. These data are similar to that obtained when the cells from these organs were labeled with Fl anti-Ig, as noted above, except in the case of Peyer's patch cells, where Fl anti-κ labels 21.4% more cells than Fl anti-μ.

Finally, the fluorescence profiles of Fl anti-μ-labeled spleen cells from 3-, 10-, 17-, and 30-day-old and adult BALB/c mice are shown in Figs. 7*a–e*. These profiles are similar to one another in that they lack a fluorescence peak. However, the relative frequency of cells with a very low-to-intermediate fluorescence intensity among the positive cells increases considerably from 51.3% at 3 days to 67.2% at 17 days (Table II). The increase in this population of cells

TABLE I
Frequency of Ig-Positive Lymphocytes Among BALB/c Spleen Cells Labeled with Fl Anti-Ig

Figure	Age	Ig-positive cells	Frequency of cells, among the Ig-positive population, with a fluorescence intensity of:	
			80–200*	200–400*
	days	*%*		
4*a*	3	29.2	39.9	36.6
4*b*	10	34.8	42.8	40.7
4*c*	17	54.0	44.2	42.1
4*d*	30	49.7	36.5	43.0
4*e*	Adult	44.0	23.2	50.8

* Fl anti-Ig-labeled lymphocytes appearing in channels 80–200 and 200–400 are designated as having a very-low density and low-to-intermediate density of surface Ig.

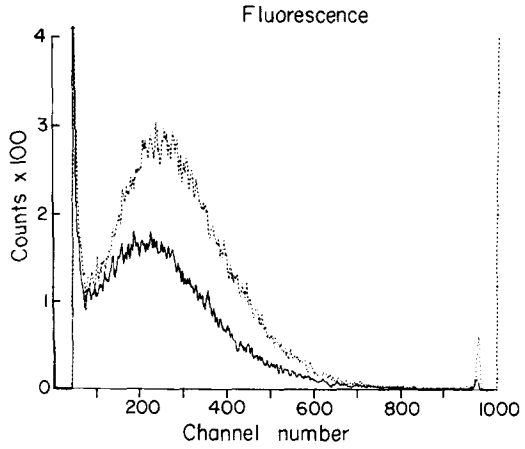

FIG. 5. Fluorescence profiles of spleen cells labeled with Fl anti-Ig from +/*nu* BALB/c (—), or *nu*/*nu* BALB/c (---) mice.

continues so that at 30 days of age these cells represent 73.4% of the total surface IgM-positive population, which is similar to the frequency of these cells in the spleen cells of adults (Fig. 7*e*). Thus, as B lymphocytes mature in the spleens of neonatal mice, the relative density of their surface IgM decreases.

Discussion

In this study, we have utilized the technology of RFMF to determine the relative density of surface Ig on B lymphocytes derived from different lymphoid organs of adult mice and from the spleens of neonatal mice in different stages of their maturation. Splenic B lymphocytes derived from adult DBA/2, BALB/c, CBA/J, C57BL/6, and C3H/J mice labeled with either Fl anti-Ig or Fl anti-κ had a fluorescence profile which was characterized by a large peak of cells with a relatively homogenous intensity of fluorescence. The cells within this peak have

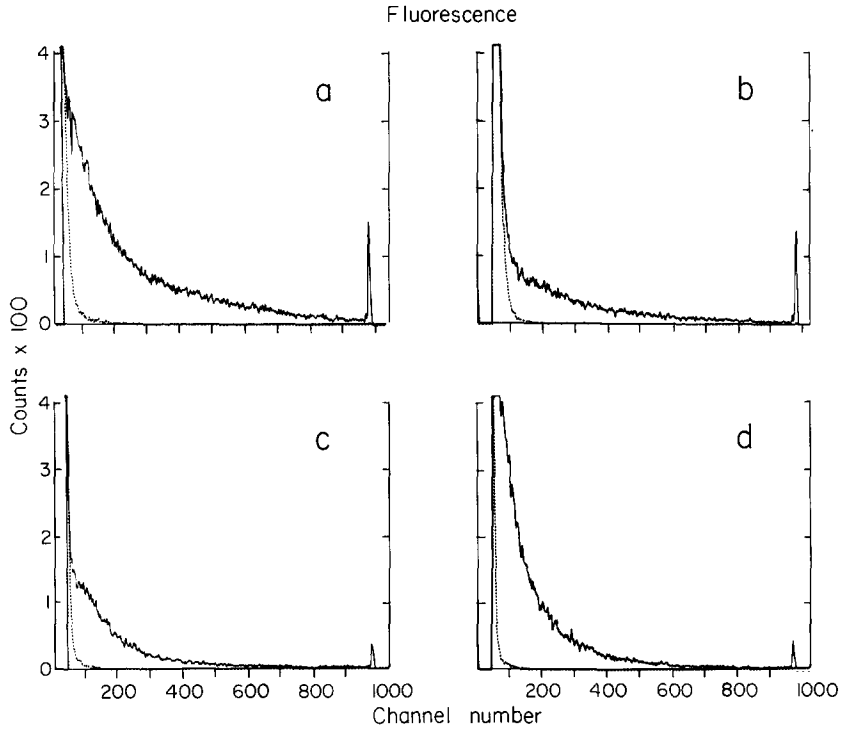

FIG. 6. Fluorescence profiles of (*a*) CBA/J spleen cells unlabeled (---) or labeled with Fl anti-μ (—), (*b*) CBA/J bone marrow cells unlabeled (---) or labeled with Fl anti-μ (—), (*c*) CBA/J lymph node cells unlabeled (---) or labeled with Fl anti-μ (—), and (*d*) CBA/J Peyer's patch cells unlabeled (---) or labeled with Fl anti-μ (—).

a low-to-intermediate density of total surface Ig. Moreover, since Fl anti-γ_2 antibody labeled only 3.8% of splenic lymphocytes, it is unlikely that cells bearing passively acquired cytophilic antibody of the IgG_2 class contribute to any extent to this peak. The characteristic profile observed after labeling adult spleen cells with Fl anti-Ig was also seen when adult spleen cells derived from either *nu/nu* mice or from mice reared under germfree conditions were examined. Thus, the development of the large, relatively homogenous population of splenic B lymphocytes, which have a low-to-intermediate density of surface Ig, appears to be independent of either antigenic stimulation or T-lymphocyte cooperation. Moreover, the absence of the low-to-intermediate fluorescence peak among adult bone marrow cells and neonatal spleen cells and its presence in adult spleen and lymph node cells suggest that the cells with a low-to-intermediate density of surface Ig represent a distinct, mature subclass of B lymphocytes.

The fluorescence profiles of Fl anti-μ-labeled cells derived from adult mouse bone marrow, adult spleen, or neonatal spleen lacked the peak seen when Fl anti-Ig-labeled adult spleen cells were examined. Nonetheless, a clear difference in the fluorescence profiles of Fl anti-μ-labeled adult and neonatal spleen was noted. On the other hand, the Fl anti-μ profiles of adult bone marrow and adult spleen were not distinguishable. Thus, the relative immaturity of adult bone

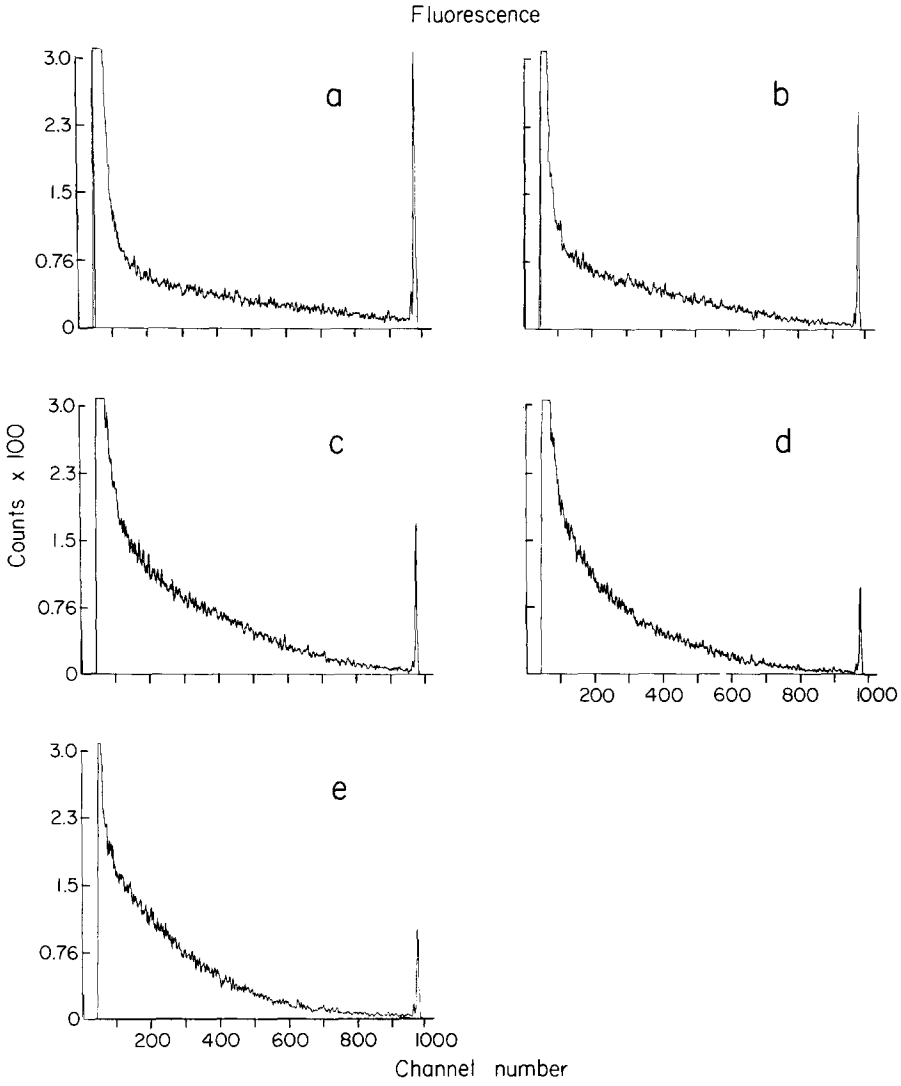

FIG. 7. Fluorescence profiles of BALB/c spleen cells labeled with Fl anti-μ from (*a*) 3-day-old, (*b*) 10-day-old, (*c*) 17-day-old, (*d*) 33-day-old, and (*e*) adult mice.

marrow cells, which is observed after comparing the fluorescence profiles of Fl anti-Ig-labeled adult bone marrow and adult spleen cells, is not detected when such cells are labeled with Fl anti-μ.

The frequency of IgM-bearing B lymphocytes within the spleen, lymph node, and bone marrow of mice is similar to the frequency of cells from these lymphoid organs detected by Fl anti-Ig or Fl anti-κ. Other authors using conventional fluorescence microscopy have reported that the frequency of IgM-bearing lymphocytes is substantially less than that of Ig-bearing B lymphocytes in these organs (6). This apparent discrepancy is most likely explained by the ability of the FACS to detect those cells with a level of fluorescence which is just above

TABLE II
Frequency of IgM-Positive Lymphocytes Among Spleen Cells from BALB/c Mice Labeled with Anti-μ

Figure	Age	IgM-positive cells	Frequency of cells, among IgM-positive cells, with a fluorescence intensity of 80–400*
	days	%	
7*a*	3	33.6	51.3
7*b*	10	35.8	59.2
7*c*	17	52.1	67.2
7*d*	30	45.1	73.4
7*e*	Adult	42.9	75.7

* Cells appearing in channels 80–400 are designated as having a very-low-to-intermediate density of surface IgM.

that of unlabeled cells and which might not be detected as Ig-positive cells using conventional microscopy. This is not an important problem with Fl anti-Ig- or Fl anti-κ-labeled spleen or lymph node cells because the relative frequency of cells with a very low level of fluorescence intensity is quite low. However, such cells represent a larger fraction of cells labeled by Fl anti-μ. On the other hand, we did observe a large difference in the frequency of cells labeled by Fl anti-κ and by Fl anti-μ in Peyer's patch cell suspensions, as has been previously reported by Guy-Grand and his co-workers (7).

A number of observations suggest that the development of the peak of splenic B lymphocytes is associated with the acquisition by these cells of the surface membrane protein which is the putative homologue of human surface IgD (subsequently referred to as IgD) (8). The appearance of the adult splenic Fl anti-Ig-labeled fluorescence profile and of an adult ratio of surface IgM to IgD occurs after the first 30 days of life and their development is seen in both germfree and *nu/nu* mice. In addition, the differences between the profiles of Fl anti-Ig-labeled bone marrow and spleen, and the similarities seen after labeling these cells with Fl anti-μ can be best explained by the absence of surface IgD on the surface of bone marrow cells, as noted by Vitetta et al. (8). The decrease in the fraction of Fl anti-Ig-labeled very-low-to-low fluorescence intensity cells and the increase in the frequency of Fl anti-μ-labeled very-low-to-low fluorescence intensity cells in developing mice is, therefore, most likely due to the acquisition of surface IgD by maturing splenic B lymphocytes which bear very-low-to-low densities of surface IgM.

It is not clear from our data if a population of B lymphocytes that exclusively expresses IgD exists in lymph node or spleen. However, if such a population is present in these organs, then it must represent a relatively small fraction of Ig-bearing B lymphocytes, as the frequency of IgM-positive cells was only slightly less than that of cells detected with Fl anti-Ig. It is possible that a population of B lymphocytes exists with a relatively high IgD-to-IgM ratio but which still bears sufficient IgM to be detected by the FACS. Of course, unanticipated cross-reactions between IgM and IgD may exist and may lead to an overestimate of

the relative frequency of IgM-bearing cells. In Peyer's patches, on the other hand, there may be a substantial number of cells which bear IgD only.

The development of a large population of splenic B lymphocytes with a low-to-intermediate density of surface Ig also coincides temporally with the acquisition of the antigens responsible for the minor lymphocyte-stimulating (Mls) mixed lymphocyte reactions and the appearance of complement receptor lymphocytes (9, 10). These data indicate that the development of Mls-defined antigens, the complement receptor, surface IgD, and large numbers of lymphocytes with a low-to-intermediate density of surface Ig serve as markers of a mature subpopulation of B lymphocytes. Moreover, they show that the acquisition of immunocompetence by B lymphocytes is not simply a function of expression of large amounts of immunoglobulin on the cell membrane.

Summary

The density of total Ig and of IgM, IgG_1, IgG_2, and IgA on the surface of adult murine splenic B lymphocytes was measured using the technique of rapid flow microfluorometry. In addition, the density of total surface Ig and of IgM on B lymphocytes derived from adult bone marrow, lymph nodes, and Peyer's patches, and from neonatal spleen was determined. Adult spleen and lymph node B lymphocytes are characterized by the presence of a large population of cells with a low-to-intermediate density of total surface Ig, which is seen as a peak in the fluorescence profiles when these cells are labeled with fluorescein-conjugated (Fl) anti-Ig. This peak is not detected when neonatal spleen or adult bone marrow are examined; the development of this peak among spleen cells occurs during the first 4 wk of life. Although the characteristic fluorescence intensity peak is not seen when adult spleen cells are labeled with Fl anti-μ, changes in the density of surface IgM do occur during the first few weeks of life and are detected as a decrease in the frequency of cells which have relatively large amounts of surface IgM. The differences seen in the fluorescence patterns of adult spleen cells labeled with Fl anti-Ig and Fl anti-μ may be due to the appearance of IgD on the surface of mature splenic B lymphocytes. This is supported by the similarity of the fluorescence profiles of adult bone marrow cells stained with Fl anti-Ig and Fl anti-μ, as the latter population of cells is reported to lack surface IgD.

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