ONTOGENY OF Ia AND IgD ON IgM-BEARING B LYMPHOCYTES IN MICE*

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The Ia antigens, coded for by the *I* region within the major histocompatibility complex *H-2*, have been identified on lymphocytes, macrophages, spermatozoa, epidermis, and undefined fetal liver cells (1, 2). Lymphoid cells that express surface Ia are predominantly B cells (3), although subpopulations of T cells may also express *I* region-determined antigens (4). It has recently been shown that Ia antigens as well as surface immunoglobulins may be involved in the regulation of T-cell and B-cell interactions in the immune response (5–7).

The relative frequencies and rates of appearance of Ia-positive cells and of Ig-bearing cells appear to coincide during ontogeny, and the majority of immunoglobulin(Ig)-bearing cells in adult spleen also express Ia (8). On the other hand, the studies of Hammerling et al (9) suggest the presence in adult mice of Ig^+ Ia^- lymphocytes which may give rise to Ig^+ Ia^+ cells. It is also known that in perinatal mice, B cells lack several surface markers, such as Fc and C3 receptors and IgD, which are found on mature B cells in peripheral tissues of adults (10, 11). The absence of such membrane receptors on recently formed or young B cells may relate to their greater susceptibility to tolerance induction by multivalent antigens (12, 13), ease of suppression by anti-immunoglobulin antibodies (14–16), and a tendency to make more IgM than IgG antibodies in clonal assays (17).

Since expression of Ia and IgD on B cells may be important in determining response patterns to antigens, we have used double immunofluorescent staining techniques to define the ontogenetic sequence of these markers on individual lymphocytes.

Materials and Methods

Animals. Inbred CBA/J and SJL/J mice, obtained from The Jackson Laboratories, Bar Harbor, Maine, were used at 2–3 mo of age for study or breeding. The first day of gestation was estimated to be the day on which a vaginal plug was found.

Cell Preparations and Tissue Culture. Cell suspensions for culture or fluorescent staining were cleared of nonviable cells and cultured with or without lipopolysaccharide (LPS) as previously described (16).

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Antisera. Anti-H-$2K^k$ (K-603) was produced by immunization of (C3H.OH × 129/Sv)F_1 hybrids with C3H/HeJ cells. This antisera had a titer in cytotoxic tests of 1:1024 with CBA/J (H-2^k) cells. The anti-Ia^k serum (K-492) was made by immunization of A.TH mice with spleen cells from congenic A.TL animals. This antiserum contained predominantly anti-IaA^k antibodies and had a titer of 1:500 in cytotoxic tests with H-2^k cells. An anti-Ia^s antiserum (K-463) was made by immunizing (A.TL × B10)F_1 mice with spleen cells from (A.TH × B10)F_1 spleen cells and had a titer of 1:128 against B10.S spleen cells.

Immunofluorescence Assays. An indirect fluorescence method was used to detect surface alloantigens. 1,000,000 viable cells were incubated at 4°C with 20 μl of neat alloantiserum, washed twice, and then stained with 20 μl of rhodamine (RITC) conjugated goat anti-mouse γ_1-chain. Cells were then stained for surface IgM by incubating the cells at 4°C in 20 μl of fluorescein (FITC) conjugated goat anti-mouse μ-chain. The preparations, characterization, and specificity of these goat anti-mouse immunoglobulin reagents have been described elsewhere (16). IgD was detected on the surface of lymphocytes by incubating cells first with rabbit anti-mouse IgD (18). The cells were washed twice and stained with goat FITC anti-rabbit IgG under capping conditions. The cells were then stained with goat RITC anti-mouse IgM at 4°C, washed, layered onto slides with a cytocentrifuge, and fixed for 15 min in 5% acetic acid in ethanol at −20°C. After washing the slides in phosphate-buffered saline, pH 7.4, fixed cells were sometimes stained for cytoplasmic IgM, before mounting with coverslips and Elvanol for examination by fluorescence microscopy (16).

Results and Discussion

Of the $sIgM^+$ B lymphocytes in fetal spleen and liver 1 day before birth only a very small fraction expressed surface Ia. As shown in Fig. 1, after birth there was a linear increase in the percentage of $sIgM^+$ cells which expressed Ia. By 9 days 95–100% of splenic $sIgM^+$ cells also stained for Ia as is the case for adult splenic B cells. The frequency of $sIgM^+$ cells expressed as a percentage of total nucleated spleen cells increased from 1 to 2% at birth and from 8 to 11% by 9 days of age. We also studied the appearance of Ia^s antigens on $sIgM^+$ B lymphocytes in spleens of developing SJL/J (H-2^s) mice. The rate of expression of Ia antigens in this strain was similar to that of Ia^k in CBA/J mice and results are not shown. Also shown in Fig. 1 is the ontogenetic appearance of H-$2K^k$ antigens on B cells as a function of age. A proportion of the earliest B cells did not carry detectable H-2^k antigens. These observations are similar to earlier results which showed that appearance of H-2 antigens on erythrocytes of 1 to 2-day old mice was delayed in some strains (19, 20). IgD-bearing cells first appeared in low numbers at 3 days after birth and by 9 days ~60% of surface (s)IgM^+ cells also expressed IgD. Adult percentages were not obtained until later (Fig. 1).

In adult bone marrow and perinatal liver, pre-B cells, identified by small amounts of cytoplasmic IgM within lymphoid cells lacking sIgM (21, 22), did not express surface Ia determinants. As in perinatal liver and spleen, a proportion (50–60%) of $sIgM^+$ B cells in adult marrow were Ia^-. It has been shown by both immunochemical (11) and direct staining techniques (22) that a large fraction of adult marrow $sIgM^+$ lymphocytes lack sIgD.

Tissue culture experiments suggested that Ia expression is a programmed differentiation event. Suspensions of spleen or liver cells from mice 1–9 days of age were cultured in medium containing fetal calf serum but no other mitogen. After 6 days in culture all $sIgM^+$ cells expressed Ia whatever the age of the animal used to establish the culture. Expression of Ia on $sIgM^+$ cells was studied serially in cultures of 15 h neonatal liver. The rate of appearance of Ia closely approximated that observed for splenic B cells in situ (results not shown).

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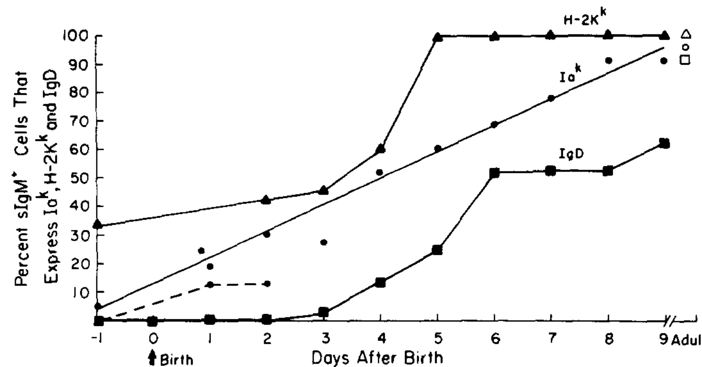

FIG. 1. Developmental relationships of H-2K^k, Ia^k, and IgD on splenic and liver B lymphocytes of fetal and neonatal CBA/J mice. Results are expressed as percentage of surface IgM-positive cells in spleen which stained with either anti-H-2K^k, —▲—; anti-Ia^k, —●—; or anti-IgD, —■—. Adult values are given on the right of the graph in open symbols. Fetal liver B lymphocytes stained with anti-Ia^k are represented by the dashed line, --●--. IgM$^+$ B lymphocytes in liver were also examined for IgD but up until 2 days of age no surface IgD$^+$ cells were detected. When adult CBA/J spleen cells were incubated with normal mouse serum or anti-Ia^s antiserum followed by RITC anti-γ1 or with RITC anti-γ1 alone, <0.5% of cells were stained.

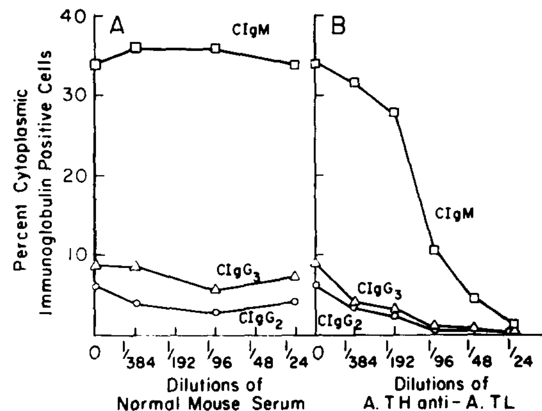

FIG. 2. Effects of normal CBA serum, (A) or A.TH anti-A.TL antiserum, (B) on the LPS induced differentiation of 1-day-old spleen cells to plasma cells synthesizing cytoplasmic IgM, —□—; IgG_2, —○—; and IgG_3 —△—. Anti-Ia serum and normal mouse serum were present at the indicated concentrations for the duration of culture.

Similarly, bone marrow from anti-μ suppressed mice, which contains pre-B cells but no sIgM$^+$ cells, gave rise to sIgM$^+$ Ia$^+$ cells after 4 days in culture (Burrows, P. Personal communication).

It has been reported that anti-Ia suppressed the proliferative response of adult mouse spleen cells to LPS (23). We have confirmed this, and have also found that LPS-induced development of IgG as well as IgM plasma cells is markedly inhibited by anti-Ia (data not shown). The results in Fig. 2 indicate that continuous exposure to anti-Ia also inhibits the LPS-dependent differentiation of newborn spleen B lymphocytes to cells containing cytoplasmic IgM and IgG. This observation together with the finding that plasma cells developing in

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nonsuppressed cultures all have cell surface Ia, suggests obligate expression of Ia in LPS-induced differentiation of $sIgM^+$ lymphocytes to plasma cells. Other experiments have indicated that Ia antigens play a critical role in induction of IgG responses to thymus-dependent antigens, but may not be expressed on all virgin IgM precursors (6–7). Our results suggest that Ia-negative precursors for IgM responses to thymus-dependent antigens are either not triggerable by LPS or acquire Ia after antigenic stimulation.

The present results demonstrating the existence in newborn tissues of a population of $sIgM^+$ Ia^- B lymphocytes, may be related to the many functional differences between newborn and adult B cells (12, 13). The rate at which B cells from young mice lose susceptibility to tolerance induction and to inhibition by anti-μ antibodies (14–16) is strikingly parallel to the rate at which baby B cells acquire Ia. The relative predominance of precursors from neonatal tissues which give rise to clones synthesizing only IgM antibodies, the frequency of which decreases with age, might also be related to the $sIgM^+$ Ia^- population of neonatal spleen cells (17). Because the ontogenetic appearance of Ia correlates more closely with functional changes occurring in B lymphocytes during the 1st-wk of life, we have stressed these relationships. However, either IgD or other B-cell receptors (e.g., C_3 and Fc) may be related to these functional changes.

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Summary

We used immunofluorescence to examine the developmental relationship of Ia and IgD on B cells. Pre-B cells in fetal liver did not express Ia. Only very few surface IgM-positive ($sIgM^+$) B cells in fetal spleen were found to be Ia^+ and were weakly stained for Ia. After birth there was a linear increase in the proportion of $sIgM^+$ spleen cells which expressed Ia, reaching ~95% by 9 days. Adult bone marrow also contains a sizeable proportion of $sIgM^+$ Ia^- cells. Unstimulated cells from fetal or newborn liver and spleen expressed Ia at the same rate in culture. Anti-Ia antisera suppressed the LPS-induced differentiation of IgM and IgG plasma cells in cultures of neonatal lymphocytes. Ia was also detected on IgM and IgG plasma cells in vitro suggesting that lipopolysaccharide (LPS)-stimulated B cells may express Ia antigens, induced by LPS, or appearing as part of normal differentiation. IgD did not appear on $sIgM^+$ cells until 3 days of age and then rose slowly to reach adult levels later than Ia antigens.

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References

1. Hammerling, G. J., B. D. Deak, C. Mauve, U. Hammerling, and H. O. McDevitt. 1974. B-lymphocyte alloantigens controlled by the I region of the major histocompatibility complex in mice. *Immunogenetics*. 1:68.
2. Delovitch, T. L., and H. O. McDevitt. 1975. Isolation and characterization of murine Ia antigens. *Immunogenetics*. 2:39.
3. Sachs, D. H., and J. L. Cone. 1973. A mouse B-cell alloantigen determined by gene(s) linked to the major histocompatibility complex. *J. Exp. Med.* 138:1289.

4. Frelinger, J. A., J. E. Niederhuber, C. S. David, and D. C. Shreffler. 1974. Evidence for the expression of Ia (H-2 associated) antigens on thymus-derived lymphocytes. *J. Exp. Med.* **104**:1273.
5. Benacerraf, B., and D. H. Katz. 1975. The histocompatibility linked immune response genes. *Adv. Cancer Res.* **21**:121.
6. Pierce, S. K., and N. R. Klinman. 1975. The allogeneic bisection of carrier-specific enhancement of monoclonal B-cell responses. *J. Exp. Med.* **142**:1165.
7. Press, J. L., N. R. Klinman, and H. O. McDevitt. 1976. Expression of Ia antigens on hapten-specific B cells. I. Delineation of B-cell subpopulations. *J. Exp. Med.* **144**:414.
8. Press, J. L. 1975. Splenic expression of Ia alloantigens during murine neonatal development. *Fed. Proc.* **34**:945.
9. Hammerling, U., A. F. Chen, and J. Abbott. 1976. Ontogeny of murine B lymphocytes: Sequence of B-cell differentiation from surface-immunoglobulin-negative precursors to plasma cells. *Proc. Natl. Acad. Sci. U. S. A.* **73**:2008.
10. Gelfand, M. C., G. J. Elfenbein, M. M. Frank, and W. E. Paul. 1974. Ontogeny of B lymphocytes. II. Relative rates of appearance of lymphocytes bearing surface immunoglobulin and complement receptors. *J. Exp. Med.* **139**:1128.
11. Abney, E. R., and R. M. E. Parkhouse. 1974. Candidate for immunoglobulin D present on murine B lymphocytes. *Nature (Lond.).* **252**:600.
12. Nossal, G. J. V., and B. L. Pike. 1975. Evidence for the clonal abortion theory of B-lymphocyte tolerance. *J. Exp. Med.* **141**:904.
13. Metcalf, E. S., and N. R. Klinman. 1976. In vitro tolerance induction of neonatal murine B cells. *J. Exp. Med.* **143**:1327.
14. Raff, M. C., J. J. T. Owen, M. D. Cooper, A. R. Lawton, M. Megson, and W. Gathings. 1975. Differences in susceptibility of mature and immature mouse B lymphocytes to anti-immunoglobulin induced immunoglobulin suppression in vitro: possible implications for B-cell tolerance to self. *J. Exp. Med.* **142**:1052.
15. Sidman, C. L., and E. R. Unanue. 1975. Receptor mediated inactivation of early B lymphocytes. *Nature (Lond.).* **257**:149.
16. Kearney, J. F., M. D. Cooper, and A. R. Lawton. 1976. B-lymphocyte differentiation induced by lipopolysaccharide. III. Suppression of B cell maturation by anti-mouse immunoglobulin antibodies. *J. Immunol.* **116**:1664.
17. Press, J. L., and N. R. Klinman. 1973. Enumeration and analysis of antibody-forming cell precursors in the neonatal mouse. *J. Immunol.* **111**:829.
18. Abney, E., I. R. Hunter, and R. M. E. Parkhouse. 1976. Preparation and characterization of an antiserum to the mouse candidate for immunoglobulin D. *Nature (Lond.).* **259**:404.
19. Möller, G., and E. Möller. 1962. Phenotypic expression of mouse isoantigens. *J. Cell. Comp. Physiol.* **60** (Suppl.):107.
20. Boubelík, M., and A. Lengerová. 1971. Genetic control of developmental expression of H-2 antigens. *In* Proc. Symp. Immunogenetics of H-2 System. A. Lengerová and M. Vojtisková, editors. Karger, AG., S. Basel, Switzerland. 85.
21. Raff, M. C., M. Megson, J. J. T. Owen, and M. D. Cooper. 1975. Early production of intracellular IgM by B-lymphocyte precursors in the mouse. *Nature (Lond.).* **259**:224.
22. Cooper, M. D., J. F. Kearney, A. R. Lawton, E. R. Abney, R. M. E. Parkhouse, J. L. Preud'homme, and M. Seligmann. 1976. Generation of immunoglobulin class diversity in B cells: A discussion with emphasis on IgD development. *Ann. Immunol. (Inst. Pasteur).* **127C**:573.
23. Neiderhuber, J. E., J. A. Frelinger, A. Coutinho, E. Dugan, and D. C. Shreffler. 1975. Effects of anti-Ia sera on mitogenic responses. I. Inhibition of the proliferative response to B cell mitogen, LPS by specific anti-Ia sera. *J. Immunol.* **115**:1672.

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