CELL SURFACE IMMUNOGLOBULIN

XI. The Appearance of an IgD-Like Molecule on Murine Lymphoid Cells During Ontogeny*

By ELLEN S. VITETTA, ULRICH MELCHER, MICHAEL McWILLIAMS,‡ MICHAEL E. LAMM,§ JULIA M. PHILLIPS-QUAGLIATA,‖ AND JONATHAN W. UHR

(From the Department of Microbiology, The University of Texas Southwestern Medical School, Dallas, Texas, 75235, and the Department of Pathology, New York University School of Medicine, New York 10016)

IgD has been found on the surface of 15% of the lymphocytes in human cord blood (1–3) and on 2–4% of peripheral blood lymphocytes in adult humans (4), although it is undetectable in fetal serum and a minor Ig class in adult serum (5). Many lymphoid cells in normal (2, 6) and leukemic (7) individuals have both IgD and IgM on their surface as shown by immunofluorescence using monospecific antisera coupled to two different fluorochromes. The possibility that IgD is passively adsorbed on IgM-bearing cells has been excluded by two observations (2): after surface IgD has been removed by capping, the cells regenerate surface IgD in the absence of IgD in the medium; and single cells which have both classes of Ig on their surface bear only one type of light chain. Cells expressing both IgD and IgG on their surface have not been found (2). Based on these observations, it has been suggested that IgD is the first receptor to appear on B lymphocytes (2).

We have recently reported that, in addition to monomeric IgM, there is an IgD-like molecule on the surface of murine splenocytes (8).[1] The molecule is disulfide-bonded, has a molecular weight of approximately 165,000 daltons as determined by acrylamide gel electrophoresis in sodium dodecyl sulfate (SDS) and contains L and H chains. The H chains have an apparent molecular weight of approximately 65,000 daltons, are antigenically distinct from μ-, γ- and α-chains, are highly susceptible to proteolysis, and appear to have a higher carbohydrate

* Supported by National Institutes of Health grants AI 09647, AI 11851, AI 09738, AI 09647, CA 08627 and grant 914 from The Council for Tobacco Research, U.S.A., Inc.

‡ Post doctoral fellow supported by the Pathology Training Program NIH grant GM 000127.

§ Career Scientist, Health Research Council of the City of New York, under Contract I-474.

‖ Research Career Development Award I-K04 AI 70653 National Institute of Allergy and Infectious Diseases.

[1] For the sake of simplicity, we will call this molecule IgD in the remainder of the paper. We consider the evidence indicating that this molecule is the murine counterpart of human IgD to be formidable but *not* conclusive. More definitive classification awaits a demonstration of antigenic cross-reactivity with human δ-chain and amino acid sequencing.

content than μ-chains. The present studies indicate that, during ontogeny, this IgD molecule appears on the surface of splenocytes after IgM and, in adult mice, is absent from bone marrow and thymus, but present on peripheral and mesenteric lymph node cells in large amounts. We suggest, therefore, that IgM is the first antigen-specific receptor on virgin B lymphocytes and that IgD represents a switch of cell surface receptor during differentiation. Lymphocytes from athymic and germfree mice bear both IgM and IgD, and it thus appears that the switch is independent of T-cell influence and may also be independent of antigenic stimulation.

Materials and Methods

Mice. nu/nu and nu/BALB/c mice were from our own colony and had been backcrossed six times with BALB/c mice (Jackson Laboratories, Bar Harbor, Maine). For each group of nu/nu mice, heterozygous and normal homozygous littermates (referred to in the text as nu/BALB/c or "littermates") were used as controls.

Germfree C3H mice (Sprague-Dawley, Inc., Madison, Wis.) were sacrificed immediately after removal from their germfree isolators. Adult BALB/c mice, ranging in age from 6 wk to 2 yr, were purchased from Jackson Laboratories and were maintained in our colony.

Antisera. Rabbit anti-μ prepared against isolated μ-chains from MOPC-104E myeloma protein (9) and rabbit anti-Ig (μ, γ, α, κ, λ) antisera were prepared, assayed, and used as previously described (9, 10). Immune complexes were precipitated with goat antirabbit Ig (11).

Preparation of [^{3}H]IgM (μ, κ). HP76 multiple myeloma cells (which secrete κ-containing 19S IgM) obtained from Dr. Noel Warner, Hall Institute, Melbourne, Australia, were maintained by serial subcutaneous passage in BALB/c mice. Cells teased from tumors 3–4 wk after transplantation were labeled with [^{3}H]leucine (50 Ci/mmol, New England Nuclear, Boston, Mass.) added to Eagle's minimal essential medium (Grand Island Biological Co., Grand Island, N. Y.) lacking leucine and containing 10% fetal calf serum at a final concentration of 5×10^6 cells/ml. Cells were labeled with 20 μCi/ml [^{3}H]leucine. After 3–5 h of incubation, the medium was concentrated and frozen at −20°C. Before use, the medium was thawed and centrifuged at 10,000 *g* for 30 min, and the [^{3}H]IgM was immunoprecipitated with anti-Ig. Washed precipitates were dissolved in 1% SDS containing 8 M urea, pH 8.4, reduced, and alkylated, and aliquots were used as internal markers (μ- and κ-chains) for electrophoresis in acrylamide gels.

Radioiodination of Lymphoid Cells. $2–10 \times 10^7$ cells from spleen, bone marrow, peripheral lymph nodes, mesenteric lymph nodes, or thymus were radioiodinated as previously described (11). Cells were washed, lysed in 0.5% Nonidet P40 (Shell Chemical Corp., New York) (NP40) and the lysates dialyzed for 16 h against phosphate-buffered saline, pH 7.3 (PBS). In instances where dialyzed lysates were stored at 4°C (for up to 24 h) before immunoprecipitation, Trasylol (Bayer Co., New York) (8, 12) was added to a final concentration of 2,000–5,000 U/ml. This precaution was taken to prevent proteolysis of Ig molecules. Acid-precipitable radioactivity was determined as previously described (11).

Immunoprecipitation. Radioiodinated Ig was precipitated from dialyzed lysates by a sandwich procedure using amounts of antisera which bound all reactive molecules (10). Precipitations were done within a period of 4 h. All precipitates were washed with cold PBS until there was no more than a 10% loss in radioactivity between sequential washes. This usually averaged four to six washes.

Acrylamide Gel Electrophoresis. Washed precipitates were stored at 4°C. Within 24 h they were dissolved at 56°C, reduced, and alkylated (11), and aliquots containing 10,000–20,000 cpm were mixed with three times that number of CPM of [^{3}H]IgM and were immediately electrophoresed for 4–17 h in sodium dodecyl sulfate-7.5% polyacrylamide gels (SDS-PAGE) at 5–15 mA/gel. The entire gel was then fractionated and the double labels were counted in a Beckman LS350 spectrometer (Beckman Instruments, Inc., Fullerton, Calif.) with discriminator settings appropriate to the two isotopes.

Results

Distribution of IgD in Lymphoid Tissues from Adult Mice. Slightly more than 50% of radioactive surface Ig on splenocytes and 74–85% on lymph node cells was IgD (Table I). IgD was absent from both bone marrow and thymus cells. The small amount of Ig on bone marrow cells appeared to be entirely IgM. No Ig was detected on thymocytes. These results indicate that IgD is absent from the central lymphoid tissues examined; it is present in higher amounts (relative to IgM) in lymph nodes than in spleen.

Appearance of IgD During Ontogeny. As seen in Table II the percent of total cell surface protein that is Ig increased as a function of age. IgM was the only class of Ig observed on splenocytes of 4-day old mice (Fig. 1 and Table II). IgD first appeared on the cell surface between 9 and 15 days of age (Table II) and increased up to 3 mo of age (Fig. 1). Between 3 mo and 2 yr of age 50–60% of radioactive surface Ig was IgD (not shown).

TABLE I

*Distribution of IgD and IgM on Cells from Murine Lymphoid Tissue**

Tissue‡	Acid-precipitable radioactivity per 10^7 cells	Percent of acid-precipitable radioactivity specifically precipitable by anti-Ig	Percent of Ig H chains that are:§	
			μ	δ
	$cpm \times 10^{-6}$			
Thymus	2.1	0.4	0	0
Bone marrow	5.4	1.2	100	0
Spleen	2.6	5.8	43	57
Mesenteric nodes	3.0	4.8	26	75
Peripheral nodes	3.2	6.7	15	85

* 80-day old BALB/c mice. This is one of two comparable experiments.

‡ Pooled cells from 10 animals.

§ Determined by measuring the area under the H-chain peaks on SDS-PAGE. No other H-chain peaks were detected.

TABLE II

*Development of Surface IgD on Splenocytes of Neonatal BALB/c Mice**

Age	Acid-precipitable radioactivity per 10^7 cells	Percent of acid-precipitable radioactivity specifically precipitable by anti-Ig	Percent of Ig H chains that are:	
			μ	δ
days	$cpm \times 10^{-6}$			
4	7.1	2.5	100	0
9	7.7	2.6	>95	0–5
15	7.5	3.4	90	10
18	7.3	5.7	72	28

* Pooled cells from three to five animals. This is one experiment of three that were done using animals of similar ages. Results from all three experiments were similar.

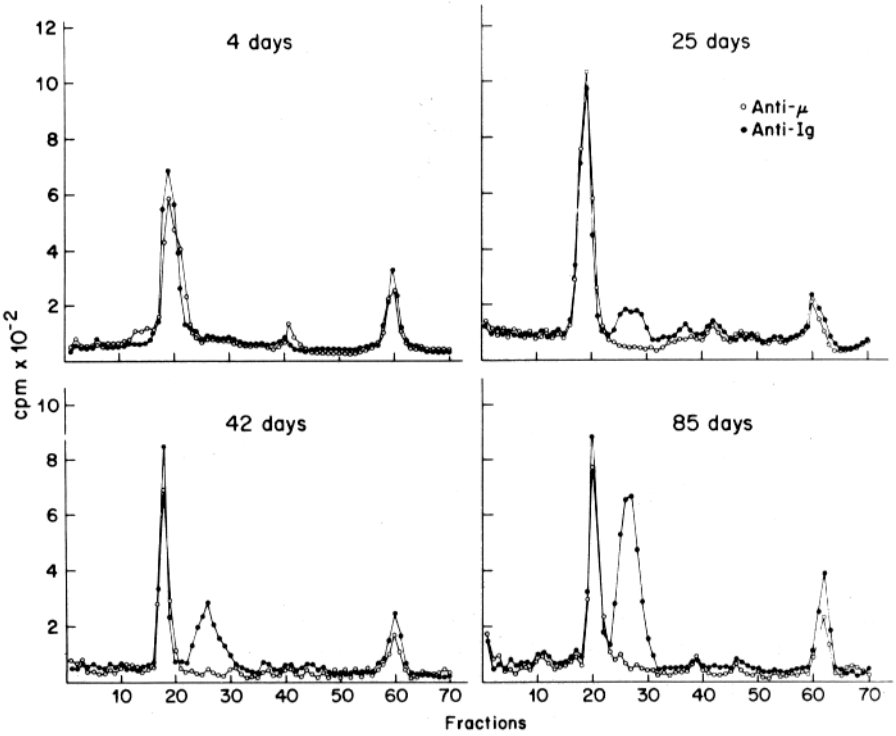

FIG. 1. Age-related appearance of IgD on the surface of BALB/c splenocytes. Separate aliquots of the lysate from radioiodinated cells were immunoprecipitated with either anti-μ or anti-Ig. The precipitates were dissolved, reduced and alkylated, and electrophoresed on 7.5% SDS acrylamide gels for 17 h. (μ-chains are in fractions 15–23, δ-chains in 22–32, and L chains in 55–65.)

We emphasize that the above quantification, which deals with radioactivity only, is a relative one. The iodinateability of different classes of Ig on the surface of lymphoid cells could be different.

Influence of the Thymus on Expression of Surface IgD. The thymus is known to influence antibody responses and/or serum levels of the IgG, IgA, and IgE classes (13–21). In order to investigate the influence of the thymus on the expression of IgD, splenocytes from nu/nu mice and their littermates were examined at various ages for the presence of IgD. The results are summarized in Table III. IgD appeared slightly later in nu/nu mice but by 20 days of age there was an equivalent proportion of IgD on the cell surface.

Two further points should be noted. First, the B cells in nu/nu mice had less radioactive Ig on the cell surface than their littermates, e.g. at 42 days of age, on an Ig/B cell basis, littermates had approximately four times as much surface Ig. Second, the growth of nu/nu animals was markedly retarded compared with littermates, and the presence and significance of chronic infection could not be readily assessed. These factors represent uncontrolled variables in the experiments.

Surface Ig on splenocytes and lymph node cells (a mixture of peripheral and

TABLE III

*Surface IgM and IgD on Splenocytes of nu/nu Mice and Normal Littermates**

Age	Acid-precipitable radioactivity per 10^7 cells		Percent of acid-precipitable radioactivity specifically precipitable by anti-Ig		Percent of Ig H chains that are:			
					μ		δ	
	nu/nu	Littermates	nu/nu	Littermates	nu/nu	Littermates	nu/nu	Littermates
days	*cpm* × 10^6							
12	1.6	3.6	3.6	3.0	100	78	0	22
20	3.8	4.3	5.0	6.2	63	64	37	36
42	3.2	3.7	4.4	8.0	70	68	30	32
85	ND	4.4	ND	10.0	ND	42	ND	58

* Littermates were a mixture of homozygous and heterozygous animals. This is one experiment of three that were done with animals of similar ages. Cells from five animals were pooled. Results of three experiments were similar.

mesenteric) from 33-day old nu/nu mice are compared in Fig. 2. Lymph node cells had IgD as their major labeled surface Ig. Considerable, but lesser, amounts of IgD were also seen in the spleen. These studies indicate that the thymus is not essential for the expression of surface IgD

Influence of Antigen on Expression of IgD. Previous studies in germfree mice have indicated that IgM is the sole Ig secreted by lymphoid cells from a variety of tissues (22). No IgG was detected. When splenocytes from such animals were examined for the presence of IgD (Table IV and Fig. 3), it was found in amounts similar to those seen in normal animals of comparable ages. There is no evidence, therefore, that antigen deprivation affected the development of surface IgD.

Discussion

The present studies indicate that IgM is readily detectable on the surface of splenocytes from 4-day old mice whereas IgD first becomes detectable 10–15 days after birth. The proportion of radioactive IgD to IgM on the surface of splenocytes increases progressively over the next several months. By 3 mo of age IgD is the preponderant cell surface immunoglobulin (in terms of radioactivity) on peripheral murine lymphoid cells. However, IgM, but not IgD, was detected on bone marrow cells. Since bone marrow cells are precursors of peripheral cells, these observations suggest that IgM rather than IgD is the primordial surface Ig. This conclusion contrasts with the hypothesis that IgD is the initial antigen-specific receptor on human B cells (2). Our studies predict that during embryogenesis in the human there is a period when only IgM will be detected on the surface of lymphocytes, a prediction compatible with the relative immunologic maturity of the human newborn compared to the mouse.

Present studies do not address the question of whether IgM and IgD molecules are on the same or different cells. However, co-capping studies in the human (2)

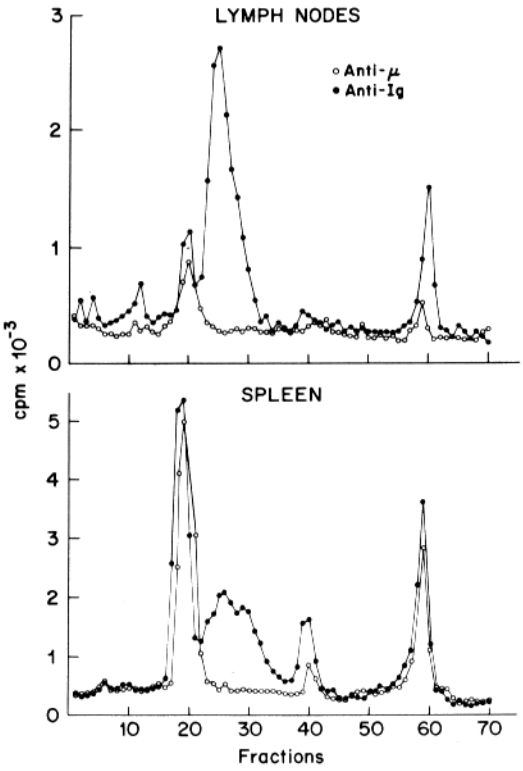

FIG. 2. Cell surface IgM and IgD on splenocytes and lymph node cells of 33-day old nu/nu mice. See legend of Fig. 1 for experimental details.

TABLE IV

*Surface IgM and IgD on Splenocytes of Germfree C3H Mice**

Age	Acid-precipitable radioactivity per 10^7 cells	Percent of acid-precipitable radioactivity specifically precipitable by anti-Ig	Percent of Ig H chains that are:	
			μ	δ
days	*cpm* $\times 10^{-6}$			
25	4.5	8.8	64	36
49	4.6	10.0	63	37
77	4.5	10.6	54	46

* Pooled cells from six animals. This was one of two experiments that were done.

have clearly demonstrated that IgM and IgD are frequently on the same cell and it is probable that a similar situation is present in the mouse. Moreover, immunofluorescence studies in mice (9) indicate that the sum of frequencies of cells bearing α-, γ-, and μ-chains is very close to the total frequency of cells bearing κ-chains in spleen and mesenteric nodes whereas there is a difference between these two values (κ being greater) in the peripheral nodes. Together with

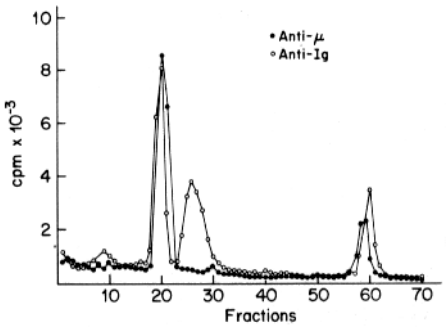

FIG. 3. Cell surface IgM and IgD on splenocytes of 25-day old germ-free C3H mice. See legend of Fig. 1 for experimental details.

the present studies, these observations suggest that IgD and IgM are found together on murine splenocytes and mesenteric node cells but on different cells in peripheral nodes. We suggest that there is a "switch" in the expression of cell surface Ig from IgM to IgD. This switch in surface Ig should be contrasted to those previously described in which there is IgM on the cell surface and simultaneously intracellular IgG, presumably destined for secretion (23). The IgD switch appears to occur in the vast majority of cells since it was the predominant class of Ig found on the peripheral lymphocyte populations studied. We emphasize, however, that our results are based entirely on incorporated radioactivity. It is possible that IgD is enzymatically labeled with ^{125}I more readily than other classes of mouse Ig. We doubt, however, that such an explanation accounts for the observation because classes of mouse Ig which are not readily detected on radioiodinated cells are easily labeled in solution, and it is very unlikely that a major portion of an Ig molecule is buried in the plasma membrane because: (*a*) the solubility of the molecule, (*b*) analogy with other membrane proteins in which only a small portion is inserted in the membrane (24–26), and (*c*) radioiodination studies (27). It is therefore more likely that these classes of Ig are not labeled because they are not present in large amounts on cells. Moreover, the studies mentioned earlier (9) indicate that more murine peripheral lymph node cells bear κ-chains than μ-, α-, and γ-chains.

It was of particular interest that IgD developed on the surface of lymphocytes of nu/nu mice in a manner generally similar to normal littermates. There was a slight delay in appearance of IgD on the surface of splenocytes of nu/nu mice but by 20 days of age and thereafter large amounts of IgD were found. These observations indicate that the thymus does not play a critical role in the presumed switch in expression of cell surface IgM to IgD. Moreover, germfree mice, 25 days old, have large amounts of IgD on the surface of their splenocytes even though IgG synthesis is not detectable (22). The simplest interpretation of these latter experiments is that the switch is also independent of antigen. However, germfree animals are exposed chronically to small amounts of antigen absorbed through the gastrointestinal tract which is presumably responsible for the significant amount of IgM synthesis present (22). It could be argued, therefore, that the switch to IgD synthesis depends upon stimulation by antigen but that the threshold is lower than that necessary to stimulate detectable IgG

synthesis and secretion. The delay in detection of cell surface IgD in normal mice until 10–15 days after birth could reflect the time taken to accumulate sufficient antigenic exposure. We favor the antigen-independent interpretation because of the lack of a distinct quantitative difference between results with germfree and conventional mice. The regulation of the IgM-to-IgD switch on the lymphocyte therefore contrasts with the switch to IgG, IgA, and IgE secretion in the plasma cell where there is considerable evidence that helper T cells and antigenic stimulation are required (13–21).

The presence of a higher proportion of IgD on the lymphocytes of peripheral lymph nodes compared to the spleen in adult mice was a consistent finding. This observation suggests that the development of IgD on splenic lymphocytes may precede emigration of such cells to peripheral lymph nodes. This concept is supported by recent observations of S. Strober (unpublished). He has described the differentiation of virgin B lymphocytes in the rat spleen. Large, slowly turning-over B cells with IgM on their surface appear to be the most primitive forms since they are the first to arise in the spleen following spontaneous recovery from sublethal X-irradiation. They appear to give rise to a dividing population and eventually to small virgin B cells found in the thoracic duct as well as the spleen. These small cells are the last to arise after recovery from irradiation and therefore appear to be the most mature cells. Provocatively, this population has several times as many cells that stain with anti-L as with anti-μ reagents. Taken together with our results, we suggest that the acquisition of IgD is a stage in the maturation of virgin B lymphocytes which takes place in the spleen (and perhaps other peripheral lymphoid organs).

The possible biological function of IgD deserves comment. Presumably, IgD and IgM molecules on the same cell have an identical specificity for antigen, that is, have identical light and heavy chain variable regions. This critical point has not been demonstrated but the presence of two classes of antibodies on the same cell with different specificities would not be readily compatible with the clonal nature of antibody secreting cells. Regardless, cells bearing both classes of Ig may not be representative of a dual recognition system but rather of a transition stage to cells bearing only IgD. (The latter cells have not as yet been described in normal lymphoid tissue, however.) Nevertheless, this idea is consistent with the concept discussed above that acquisition of IgD is a late development in differentiation of B cells. The manner in which cell surface IgD might alter the function of B lymphocytes (formerly coated with IgM) is unknown. The IgM in its monomeric form probably functions univalently with macromolecular antigens (28). If surface IgD is divalent for specific antigen, then the mature B cell's capacity to bind and retain antigen may be increased. This event could reflect a change in the antigenic threshold for stimulation. Thus, IgD-bearing cells might be more easily stimulated and also less easily tolerized.

Summary

An Ig molecule containing L chains and H chains similar to human δ-chains has been detected on the surface of radioiodinated murine lymphoid cells. Newborn mice have only IgM on their splenocytes. Between 10 and 15 days, the IgD-like

molecule appears and increases in amount until 3 mo of age, when it is the predominant cell surface Ig in terms of radioactivity. IgD is found only in peripheral lymphoid tissues and is present in larger amounts on peripheral lymph node cells (approximately 85% of surface Ig) than on splenocytes (approximately 50%). IgD is also present in comparable amounts on cells from both nu/nu and germfree mice, indicating that its expression may be independent of both thymic influence and antigenic stimulation. These studies suggest that there is a switch from cell surface IgM to IgD that occurs during differentiation of virgin B lymphocytes in the spleen.

We acknowledge the excellent technical assistance of Mr. Henry Siu, Mr. Yuen Chinn, Mrs. Mary Ann Edgar, and Ms. Roberta Summers. We are grateful to Dr. S. Strober for allowing us to see a preprint of his manuscript.

Received for publication 18 September 1974.

References

1. Rowe, D. S., K. Hug, W. P. Faulk, J. N. McCormick, and H. Gerber. 1973. IgD on the surface of peripheral blood lymphocytes of human newborns. *Nat. New Biol.* **242:**155.
2. Rowe, D. S., K. Hug, L. Forni, and B. Pernis. 1973. Immunoglobulin D as a lymphocyte receptor. *J. Exp. Med.* **138:**965.
3. Knapp, W., R. L. H. Bolhuis, J. Radl, and W. J. Hijmans. 1974. Independent movement of IgD and IgM molecules on the surface of individual lymphocytes. *J. Immunol.* **111:**1295.
4. Van Boxel, J. A., W. E. Paul, W. D. Terry, and I. Green. 1972. IgD-bearing human lymphocytes. *J. Immunol.* **109:**648.
5. Spiegelberg, H. 1972. IgD. *In* Contemporary Topics in Immunochemistry. F. P. Inman, editor. Plenum Publishing Corp., New York. **1:**165.
6. Fu, S. M., R. J. Winchester, and H. G. Kunkel. 1974. Occurrence of surface IgM, IgD and free L chains on human lymphocytes. *J. Exp. Med.* **139:**451.
7. Kubo, R. T., H. M. Grey, and B. Pirofsky. 1974. IgD: a major immunoglobulin on the surface of lymphocytes from patients with chronic lymphatic leukemia. *J. Immunol.* **112:**1952.
8. Melcher, U., E. S. Vitetta, M. McWilliams, M. E. Lamm, J. M. Phillips-Quagliata, and J. W. Uhr. 1974. Cell surface immunoglobulin X. Identification of an IgD-like molecule on the surface of murine splenocytes. *J. Exp. Med.* **140:**1427.
9. McWilliams, M., M. E. Lamm, and J. M. Phillips-Quagliata. 1974. Surface and intracellular markers of mouse mesenteric and peripheral lymph node and Peyer's patch cells. *J. Immunol.* **113:**1326.
10. Vitetta, E. S., J. W. Uhr, and E. A. Boyse. 1973. Immunoglobulin synthesis and secretion by cells in the mouse thymus that do not bear θ antigen. *Proc. Natl. Acad. Sci. U.S.A.* **70:**834.
11. Vitetta, E. S., S. Baur, and J. W. Uhr. 1970. Cell surface immunoglobulin II. Isolation and characterization of immunoglobulin from mouse splenic lymphocytes. *J. Exp. Med.* **134:**242.
12. Moroz, C., and Y. Hahn. 1973. Cell surface immunoglobulin of human thymus cells and its biosynthesis *in vitro*. *Proc. Natl. Acad. Sci. U.S.A.* **70:**3716.
13. Pritchard, H., J. Riddaway, and H. S. Micklem. 1973. Immune responses in congenitally thymus-less mice. II. Quantitative studies of serum immunoglobulins,

the antibody response to sheep erythrocytes and the effect of thymus allografting. *Clin. Exp. Immunol.* **13**:125.

14. Perey, D. Y. E., and J. Bienenstock. 1973. Effects of bursectomy and thymectomy on ontogeny of fowl IgA, IgG and IgM. *J. Immunol.* **111**:633.
15. Hamaoka, T., D. H. Katz, and B. Benacerraf. 1973. Hapten-specific IgE antibody responses in mice. *J. Exp. Med.* **138**:538.
16. Tada, T., K. Okumura, and M. Taniguchi. 1972. Regulation of homocytotropic antibody formation in the rat. VII. Carrier functions in the anti-hapten homocytotropic antibody response. *J. Immunol.* **108**:1535.
17. Kishimoto, T., and K. Ishizaka. 1972. Regulation of antibody response *in vitro*. III. Role of hapten-specific memory cells and carrier-specific helper cells on the distribution of anti-hapten antibodies in IgG, IgM and IgE classes. *J. Immunol.* **109**:612.
18. Katz, D. H., and B. Benacerraf. 1972. The regulatory influence of activated T cells on B cell responses to antigen. *Adv. Immunol.* **15**:1.
19. Okumura, K., and T. Tada. 1971. Regulation of homocytotropic antibody formation in the rat. III. Effect of thymectomy and splenectomy. *J. Immunol.* **106**:1019.
20. Tada, T., and K. Okumura. 1971. Regulation of homocytotropic antibody formation in the rat. V. Cell cooperation in the anti-hapten homocytotropic antibody response. *J. Immunol.* **107**:1137.
21. Crewther, P., and N. L. Warner. 1972. Serum immunoglobulins and antibodies in congenitally athymic (nude) mice. *Aust. J. Exp. Biol.* and *Med. Sci.* **50**:625.
22. Vitetta, E. S., I. Grundke-Iqbal, K. Holmes, and J. W. Uhr. 1974. Cell surface immunoglobulin VII. Synthesis, shedding and secretion of immunoglobulin by lymphoid cells of germ-free mice. *J. Exp. Med.* **139**:862.
23. Pernis, B., L. Forni, and L. Amante. 1971. Immunoglobulins as cell receptors. *Ann. N. Y. Acad. Sci.* **190**:420
24. Schwartz, B. D., K. Kato, S. E. Cullen, and S. G. Nathenson. 1973. *H-2* histocompatibility alloantigens. Some biochemical properties of the molecules solubilized by NP-40 detergent. *Biochemistry.* **12**:2157.
25. Segrest, J. P., I. Kahane, R. L. Jackson, and V. T. Marchesi. 1973. Major glycoprotein of the human erythrocyte membrane: evidence for an amphipathic molecular structure. *Arch. Biochem. Biophys.* **155**:167.
26. Strittmatter, P., M. J. Rogers, and L. Spatz. 1972. The binding of cytochrome b_5 to liver microsomes. *J. Biol. Chem.* **247**:7188.
27. Baur, S., I. Schenkein, and J. W. Uhr. 1972. Cell surface immunoglobulin I. Isolation and characterization of immunoglobulin from murine myeloma cells. *J. Immunol.* **108**:748.
28. Metzger, H. 1970. Structure and function of γM macroglobulins. *Adv. Immunol.* **12**:57.