CELL SURFACE IMMUNOGLOBULIN

X. Identification of an IgD-Like Molecule on the Surface of Murine Splenocytes*

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μ-chains from radioiodinated surface immunoglobulin (Ig) of murine splenocytes are markedly heterogeneous as determined by their mobility on sodium dodecyl sulfate (SDS) electrophoresis in 5% acrylamide gels (1). We considered the possibility that the heterogeneity is dependent on differing carbohydrate contents of these chains. To explore this possibility, chains were electrophoresed in gels of higher acrylamide concentration in order to reduce the effect of carbohydrate moieties on mobility (2). These studies have led to the identification of two classes of Ig on the surface of murine splenocytes. One is immunoprecipitable with anti-μ and has a heavy chain which coelectrophoreses with secreted-μ. The other is not specifically immunoprecipitable with antisera raised against purified μ-, γ-, or α-chains and is smaller in size than μ-chain. Its properties are similar to those of human δ-chain (3).

Materials and Methods

The antisera used have been previously described. They include rabbit anti-μ, anti-α, and anti-γ raised against purified heavy (H) chains (4), anti-Ig (anti-μ, γ, α, κ, λ) (5), anti-α,κ and as a control anti-ϕX 174. The anti-α,κ serum was raised against purified TEPC-15 IgA and contained antibodies only to α and κ as assessed by its reactivity with myeloma proteins of different classes. Since the monospecific anti-α (4) also prepared from TEPC-15 IgA did not react with spleen cell Ig and the anti-α,κ brought down the same radioactive molecules as multispecific anti-Ig, the anti-α,κ was used as an anti-κ reagent (called anti-κ). Immune complexes were precipitated with goat antirabbit Ig (5).

^{125}I-labeled surface and ^{3}H-tyrosine-labeled secreted Ig were obtained as previously described (1, 5) from the splenocytes of 6- to 12-wk old BALB/c mice. Preliminary experiments indicated the necessity of inhibiting proteolysis. This was accomplished by performing manipulations rapidly at 4°C. In addition, Trasylol (Bayer Co.) (6), an inhibitor of proteolysis, was employed in some experiments but its use was not essential. Dissolved precipitates were reduced and alkylated and electrophoresed in SDS-polyacrylamide gels (PAGE) (5). Secreted [^{3}H]Ig was reduced and alkylated and run as an internal marker in all gels (1). Gel fractions were counted in a Beckman Spectrometer LS 350 (Beckman Instruments, Inc., Fullerton, Calif.) at the appropriate discriminator settings for ^{125}I-labeled and ^{3}H-labeled samples.

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Results

Fig. 1 shows the results of electrophoresis in SDS 7.5% acrylamide gels of H and light (L) chains of reduced and alkylated surface Ig labeled with ^{125}I. Two ^{125}I-labeled peaks were resolved in the H-chain area, one of which coelectrophoresed with marker μ-chain, and another which migrated further into the gel. The faster peak was not observed in the [^{3}H]Ig secreted by splenocytes. The control immunoprecipitate showed no peaks in the H- or L-chain areas (not shown in Fig. 1). Two membrane H-chain peaks could also be resolved in 10% and 12.5% acrylamide gels, but not in 5% gels. These results suggest that the faster moving protein has a lower molecular weight but more carbohydrate than

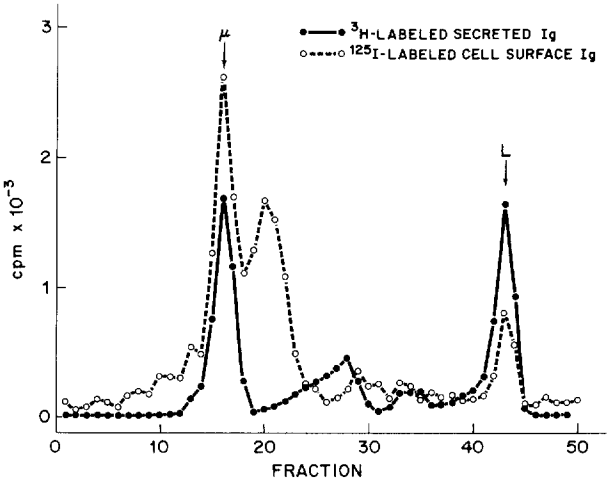

FIG. 1. SDS-PAGE of [^{125}I]splenocyte surface Ig and [^{3}H]tyrosine-labeled Ig secreted by splenocytes. Anti-Ig was used to form precipitates which were reduced and alkylated before electrophoresis on 7.5% acrylamide gels for 4 h.

μ-chain so that in low concentration gels, their mobilities are similar. This interpretation is further supported by the observation that surface and secreted IgM are less dense than the IgD-like molecule as assessed in cesium chloride density gradients (Melcher and Uhr, unpublished observations).

A lysate of ^{125}I-surface-labeled cells was precipitated with antisera to different H chains using amounts of antisera to Ig chains that precipitated maximal amounts of radioactivity (Table I). The total of separate immunoprecipitations with anti-μ, -α, and -γ was only 57% of the total membrane Ig precipitated by anti-κ. Analysis of the anti-γ precipitate by SDS-PAGE revealed the same two H-chain peaks seen in Fig. 1 but markedly reduced in area (10–15%). This finding suggests that the antiserum has anti-V_H activity. This explanation is supported by the inability of purified κ-chains to absorb this activity from the anti-γ. Similar analysis of the anti-α precipitate revealed no detectable peaks. The amount of anti-μ used in this experiment (Table I) was shown to be saturating by an additional criterion, i.e., addition of more anti-μ to the supernate of an anti-μ precipitate did not bring down more radioactivity than did addition of a control antiserum. When rabbit anti-κ was used to precipitate the supernate of an anti-μ

TABLE I

*Precipitation of Cell Surface Ig from BALB/c Splenocytes**

Antiserum	% of cell surface Ig‡ immunoprecipitated
anti-κ	100
anti-μ	40
anti-γ	10
anti-α	7

* Each aliquot contained 4.3×10^6 acid precipitable cpm.

‡ Radioactivity in anti-ϕX precipitate (control) was subtracted from radioactivity in the specific precipitate. Anti-κ was taken as 100% (1.3×10^5 cpm).

precipitate, 48% of the membrane Ig radioactivity was recovered, which accounted for virtually all the Ig not precipitated in the first anti-μ precipitation. This near quantitative recovery of membrane Ig in the sequential immunoprecipitations was only obtained if steps were taken to minimize proteolysis during the first immunoprecipitation. Without such precautions Ig fragments or no membrane Ig was recovered from the supernate of the anti-μ precipitate. The Ig fragments consisted primarily of H-L dimers. After reduction and alkylation of the fragments, an L-chain peak and a wide H-chain peak were seen, suggesting varying degrees of degradation of the H chain.

The sequential precipitation with anti-μ followed by anti-κ was used to prepare membrane IgM and the non-IgM membrane Ig for analysis of molecules by SDS-PAGE. When the unreduced anti-μ precipitate was analyzed, a single major peak corresponding to 190,000 daltons was obtained. Fig. 2 A shows the distribution of radioactivity from the reduced and alkylated anti-μ precipitate. The H and L chains coelectrophoresed with marker μ and L chains of secreted IgM. Analysis of the unreduced anti-κ precipitate from the anti-μ supernate revealed a single broad peak of 160–170,000 daltons. The reduced and alkylated precipitate revealed the fast moving H chain as well as L chain (Fig. 2 B). In this case, the H chain has apparently already been partially degraded, resulting in a broad peak with shoulders. The apparent mol wt of the slowest moving H-chain peak was 65,800 $\pm$ 1,800 daltons. Little or no membrane H chain was found in the region of ^{3}H-labeled secreted μ-chain.

Discussion

The above experiments indicate that there is an additional and hitherto unrecognized class of Ig on the surface of murine splenocytes. The results may explain the finding that in some murine lymphoid organs fluoresceinated anti-κ stains more cells than the total number stained by anti-μ, -γ, and -α (4). The H chain of this new class of Ig has the following properties: (*a*) A molecular weight (approximately 65,800 daltons) between those of μ- and γ-chains. (*b*) Not specifically, quantitatively, precipitable by anti-μ, -α, or -γ. (*c*) Probably a higher carbohydrate content than μ-chain to account for the greater effect of

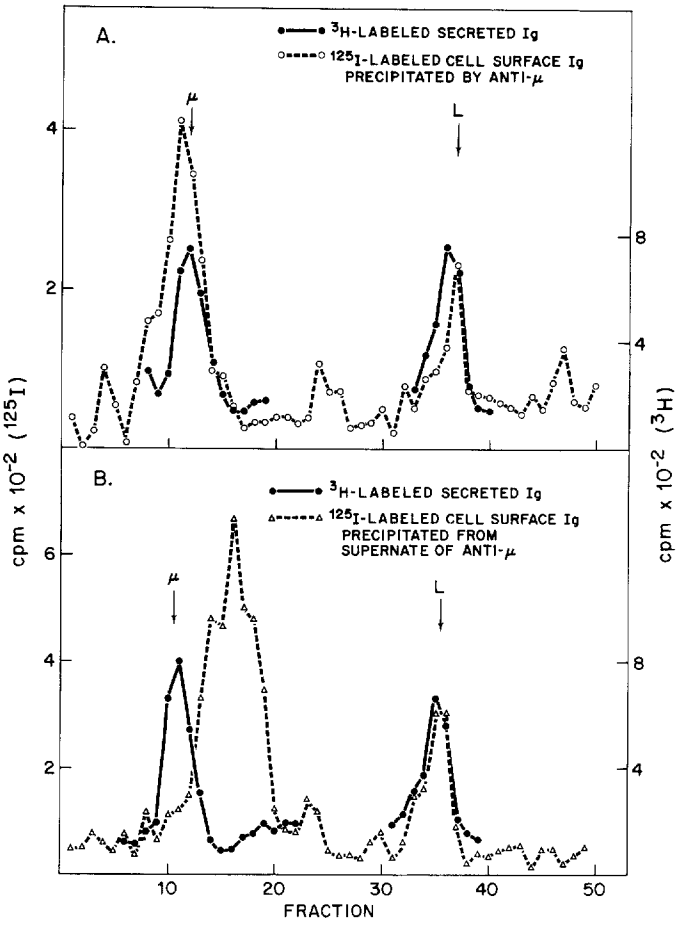

FIG. 2. SDS-PAGE of [^{125}I]splenocyte surface IgM (A) and non-IgM (B) with [^{3}H]tyrosine-labeled Ig secreted by splenocytes. The first precipitation (A) was performed with anti-μ and the precipitation of the supernate (B) performed with anti-κ. Precipitates were reduced and alkylated before electrophoresis on 10% acrylamide gels for 5 h.

changing acrylamide concentration on mobility of the new chain compared to μ-chain. (*d*) Easily degradable after cell lysis. (*e*) Not secreted by murine splenocytes in detectable amounts. The possibility that this new class of Ig is secreted and then rapidly degraded has not been excluded. These properties are shared with human δ-chain. Thus, recent mol wt determinations of human δ-chain are between 60,000 and 70,000 daltons (7, 8). Human δ-chain contains 15–16% carbohydrate whereas μ-chain contains 13–14% (3). Human IgD is unstable (3) and moreover, it is present in human sera in very low concentrations as compared to IgM, IgG, and IgA (3), but it is a major membrane Ig of human peripheral blood lymphocytes (9, 10). For these reasons, we suggest that the H chain described in this report is the murine counterpart of human δ-chain. The possibility that this H chain represents a μ-chain which is smaller in size and noncross-reactive with antibody to MOPC 104E μ-chain is unlikely.

The failure to detect the IgD-like molecule in earlier work using radioiodinated

cells (references 5 and 11–13, and footnote 1) is probably due to the marked susceptibility of δ-chain to proteolysis, the similarity of its mobility in 5% SDS gels to μ-chain, and to the findings subsequent to this study that IgM is the sole Ig on splenocytes of very young animals and the putative IgD appears in appreciable amounts only later in development.[2]

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References

1. Melcher, U., and J. W. Uhr. 1973. An electrophoretic difference between surface and secreted IgM of murine splenocytes. *J. Exp. Med.* **138**:847.
2. Segrest, J. P., R. L. Jackson, E. P. Andrews, and V. T. Marchesi. 1971. Human erythrocyte membrane glycoprotein: a re-evaluation of the molecular weight as determined by SDS polyacrylamide gel electrophoresis. *Biochem. Biophys. Res. Commun.* **44**:390.
3. Spiegelberg, H. 1972. Gamma D Immunoglobulin. *Contemp. Top. Immunochem.* **1**:165.
4. 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.* In press.
5. Vitetta, E. S., S. Baur, and J. W. Uhr. 1971. Cell surface immunoglobulin II. Isolation and characterization of immunoglobulin from mouse splenic lymphocytes. *J. Exp. Med.* **134**:242.
6. 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.
7. Perry, M. B., and C. Milstein. 1970. Interchain bridges of human IgD. *Nature (Lond.).* **228**:934.
8. Spiegelberg, H. L., J. W. Prahl, and H. M. Grey. 1970. Structural studies of human γ D myeloma protein. *Biochemistry* **9**:2115.
9. VanBoxel, J. A., W. E. Paul, W. D. Terry, and I. Green. 1972. IgD bearing human lymphocytes. *J. Immunol.* **109**:648.
10. Fu, S. M., R. J. Winchester, and H. G. Kunkel. 1974. Occurrence of surface IgM, IgD, and free light chains on human lymphocytes. *J. Exp. Med.* **139**:451.
11. Grey, H. M., R. T. Kubo, and J. C. Cerottini. 1972. Thymus-derived (T cell) immunoglobulins. Presence of a receptor site for IgG and the absence of large amounts of buried Ig determinants on T cells. *J. Exp. Med.* **136**:1323.
12. Marchalonis, J. J., R. E. Cone, and J. L. Atwell. 1972. Isolation and partial characterization of lymphocyte surface immunoglobulins. *J. Exp. Med.* **135**:956.
13. Kennel, S. J., B. C. Del Villano, and R. A. Lerner. 1973. Approaches to the quantitation and isolation of plasma membrane associated immunoglobulin. *In* Immune Response at the Cellular Level. T. Zacharia, editor. Academic Press, Inc., New York. In press.
14. Lisowska-Bernstein, B., A. Rinuy, and P. Vassalli. 1973. Absence of detectable IgM in enzymatically or biosynthetically labeled thymus-derived lymphocytes. *Proc. Natl. Acad. Sci. U. S. A.* **70**:2879.

[1] Lisowska-Bernstein et al. (14) have described an H chain on murine splenocytes which migrates further in 10% gels than μ-chain but was specifically precipitable with anti-μ.

[2] Vitetta, E. S., U. Melcher, M. McWilliams, J. Phillips-Quagliata, M. E. Lamm, and J. W. Uhr. Manuscript in preparation.