Brief Definitive Report

PRODUCTION OF PREDOMINANTLY POLYMERIC IgA BY HUMAN PERIPHERAL BLOOD LYMPHOCYTES STIMULATED IN VITRO WITH MITOGENS*

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IgA molecules present in external secretions differ from those found in human serum in their molecular forms and their subclass distribution. At least 90% of serum IgA occurs in monomeric form; in contrast, IgA found in external secretions is almost exclusively polymeric (1). Whereas ~90% of serum IgA molecules belong to the IgA1 subclass, the IgA molecules found in external secretions are equally represented by IgA1 and IgA2 (2–4). Secretory component (SC), a glycoprotein shown to bind in vitro only to polymeric forms of IgA and IgM (5, 6), has been used as a probe to detect cells that contain polymeric IgA. SC binds to a significantly higher number of intestinal lamina propria IgA plasma cells than to IgA-producing cells in bone marrow (7, 8).

The cells that eventually produce IgA for external secretions may undergo a unique migratory pattern. From the gut- and bronchus-associated lymphoid tissues, the precursor cells migrate to the thoracic duct, enter the circulation, and home to distant mucosal tissues and secretory glands, where they differentiate into IgA-producing plasma cells (9–12). The demonstration of circulating cells that have a potential to produce polymeric IgA with a subclass distribution characteristic of that known to exist in external secretions would support the validity of this specialized migratory route. We report that the IgA produced in vitro by peripheral blood lymphocytes (PBL) stimulated with various mitogens is predominantly polymeric, and the cells that produce IgA exhibit an equal distribution of IgA1 and IgA2 subclasses.

Materials and Methods

Immunoglobulins and J Chain. Polymeric IgM^{Dau}, polymeric IgA^{Fel}, monomeric IgA^{Pet}, IgG^{De}, and J chain were isolated as described previously (13).

Antisera. Antisera for heavy chains of IgG, IgM, and IgA, and antisera against J chain were prepared in goats and rabbits, and the specificity was ascertained by radioimmunoassay (RIA). Monoclonal hybridoma antibodies specific for IgA subclasses were prepared as previously described (14).

RIA. Purified myeloma proteins were labeled with carrier free $Na^{125}I$ (New England Nuclear, Boston, Mass.) using the chloramine T method (15). In the RIA, dilutions of purified

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standard proteins or of unknowns were added to polypropylene tubes with 1 ng of radiolabeled protein and the amount of specific antibody capable of precipitating 40% of the radiolabeled protein (15). *Staphylococcus aureus* (Cowan I strain ATCC-12598) or rabbit anti-goat IgG (in the IgG RIA only) was added to each tube and the radioactivity of the precipitates was measured. For quantitation of J chain, samples were fractionated by gel filtration, then reduced with 10 mM dithiothreitol and alkylated with 21 mM iodoacetamide in 0.2 M Tris, pH 8.4.

Immunofluorescence. $F(ab')_2$ fragments of monospecific antibodies to human IgA, IgM, and IgG were conjugated to fluorescein isothiocyanate or tetramethylrhodamine isothiocyanate (13). An indirect immunofluorescence staining technique was used for IgA subclass determination. Slides were examined for cytoplasmic immunoglobulin positive (cIg+) cells (13).

Culture Conditions. PBL were prepared and cultured as previously described (13). One of the following mitogens was added to PBL cultures at the indicated final concentrations: 10 μl/ml pokeweed mitogen (PWM; Grand Island Biological Co., Grand Island, N. Y.); 100 μg/ml *Escherichia coli* 0111:B4 lipopolysaccharide W (LPS; Difco Laboratories, Detroit, Mich.); or Epstein-Barr virus (EBV; obtained from supernates of the EBV-positive marmoset monkey cell line, B95-8).

Column Chromatography. PBL supernates, colostrum, and serum were fractionated by molecular gel filtration on a column (1.6 cm × 48 cm) of Ultrogel AcA 22 (LKB Instruments, Inc., Rockville, Md.) equilibrated in PBS, and calibrated with ^{125}I-labeled polymeric IgM, polymeric IgA, monomeric IgA, and IgG. For chromatography in dissociating buffer (16), the columns were equilibrated and recalibrated in 0.1 M sodium acetate buffer, pH 4.1, and the samples were dialyzed against the same buffer before chromatography.

Results

Immunoglobulin Production by PWM-Stimulated PBL. PBL from normal individuals were cultured with PWM and harvested every 24 h for 8 d. The cIg+ cells increased from <0.5% at 24 h to >16% at 192 h, with the largest increase occurring between days 3 and 5 of culture. A similar increase could be seen in the concentrations of immunoglobulins in the cell-free culture media. Large variations among individuals were observed both in the absolute amount of immunoglobulins produced (IgG, 675–3,125 ng/ml; IgM, 643–1,875 ng/ml; IgA, 142–975 ng/ml) and in the percentage of cIg+ cells (8.4–33%) on day 7.

Molecular Forms of Immunoglobulins Produced by PWM-Stimulated PBL. Supernates from 8-d cultures of PWM-stimulated PBL were pooled and fractionated on an Ultrogel AcA 22 column. The elution positions of IgM and IgG in the supernates corresponded to those obtained with radiolabeled standard IgM and IgG. A biphasic elution profile was obtained for IgA from culture supernates. The larger proportion eluted in a position that corresponded to the polymeric IgA standard (Fig. 1 A, vertical line 2).

To verify that the larger molecular weight supernate IgA was truly polymeric, fresh supernates were dialyzed against 0.1 M sodium acetate buffer, pH 4.1, and applied to a column that was equilibrated and recalibrated in the same buffer. Chromatography of supernate IgA at acid pH again revealed a predominance of the polymeric form (Fig. 1 B) and demonstrated that the IgA was not dissociated under conditions that might lead to the disaggregation of noncovalently associated monomers (16). Supernate immunoglobulins were also examined for J chain, which is covalently associated with both polymeric IgA and IgM. Supernates were fractionated as in Fig. 1 A; individual fractions were then reduced, alkylated, and assayed for J chain present in fractions that contained IgM and polymeric IgA (Fig. 1 C). Finally, supernates were reduced and alkylated before molecular filtration, and the resultant fractions were

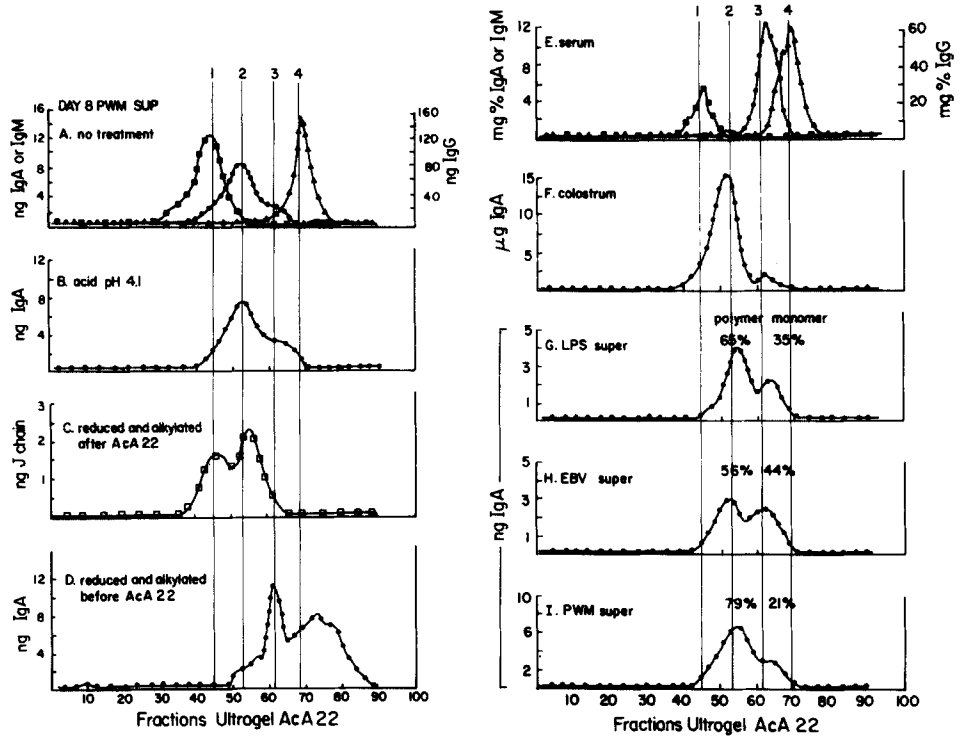

FIG. 1. Elution profiles of immunoglobulins produced by mitogen-stimulated PBL. (A) Day 8 supernate (SUP) assayed for IgM (■), IgA (●), and IgG (▲) by RIA. Aliquots of this supernate were (B) dialyzed against 0.1 M sodium acetate buffer, pH 4.1; (C) separated on Ultrogel AcA 22 column, each fraction was then reduced, alkylated, and assayed for J chain (□) by RIA; or (D) reduced and alkylated, then fractionated and assayed for IgA. (E) depicts the elution profile of immunoglobulins present in normal human serum. The elution profile of IgA in diluted (1:100 in PBS) and defatted (centrifugation) colostrum is shown in graph (F). Compare elution positions of IgA in graphs (A), (E), and (F). Supernates (super) from LPS (G), EBV (H), and PWM (I) -stimulated PBL were taken after culturing 1×10^6 cells/ml for 7 d. The percent figures represent the estimated amount of polymeric and monomeric IgA present. Small numbers on top of the vertical lines identify ^{125}I-labeled marker proteins used to standardize the Ultrogel AcA 22 column: (1) polymeric IgM, (2) polymeric IgA, (3) monomeric IgA, and (4) IgG. The curves depicted are based on the absolute content of assayed immunoglobulin. For clarity of presentation only every second or third point is shown.

assayed for IgA. As expected, the IgA elution pattern had shifted toward a predominance of monomeric IgA and smaller molecular weight components (Fig. 1 D). Therefore, the high molecular weight IgA in supernates was a true polymeric immunoglobulin, containing J chain.

To establish whether the relative proportions of monomeric and polymeric IgA produced in PBL cultures varied with time in culture, supernates were collected at days 3, 5, and 7 after PWM stimulation. On day 3, ~60% of the total IgA was present in a polymeric form, on day 5, ~70%, and by day 7, ~80% of the IgA was polymeric.

IgA Production in PBL Stimulated with LPS and EBV. LPS induced the production of polymeric and monomeric IgA in proportions similar to those found when PWM was used (Fig. 1 G and I). Approximately half of the IgA present after EBV stimulation of PBL was polymeric (Fig. 1 H). Despite variations in the polymer:monomer ratio of

TABLE I

*IgA1 and IgA2 Subclasses in PWM-Stimulated PBL**

Day after PWM stimulation	IgA1		IgA2	
	M.G.	C.O.	M.G.	C.O.
	%		%	
3	67‡	60	29	22
4	51	55	46	39
5	46	ND§	53	ND
6	38	50	59	49
7	36	38	60	55

* At least 1,000 cells were counted/slide to determine percentage of positive cells. Representative data from two individuals (M.G. and C.O.) are shown.

‡ Numbers represent percent of total IgA+ cells that were also positive for IgA1 (or IgA2).

§ ND, not determined.

IgA obtained when different mitogens were used, all three mitogens induced IgA production in ratios not typical of serum IgA (Fig. 1 E).

IgA Subclass Distribution in PWM-stimulated PBL. Because the IgA subclass distribution is also distinct for secretions as compared with serum, subclass characterization was performed on PBL cultures at various times after PWM stimulation with the use of hybridoma antibodies specific for $\alpha 1$ or $\alpha 2$ chains (Table I). Initially, IgA1 positive cells outnumbered IgA2 positive cells by 2:1, but on day 5 the distribution of IgA subclasses was approximately equal (46% IgA1, 53% IgA2), and on day 7 the number of IgA2 positive cells exceeded those producing IgA1.

Discussion

Results of the present study indicate that mitogen-stimulated B-lymphocytes from peripheral blood of normal individuals produce IgA predominantly in a polymeric form. In addition, PWM-stimulated PBL expressed IgA1:IgA2 ratios that are similar to those found in secretory tissues, where ~60% of cells produced IgA1 and 40% IgA2 subclasses (17). These values contrast with values observed in peripheral lymph nodes and normal bone marrow where 85 and 88% produced IgA1, respectively (6, 17).

Several attempts have been made to establish the source of polymeric and monomeric IgA in serum and secretions. Analyses of the intraluminal and venous return fluids obtained by perfusion of explants of human large and small intestines revealed that ~60% of IgA in venous return perfusate was present in a polymeric form (18). Immunofluorescence studies of human intestinal mucosa indicated that ~50% of cytoplasmic IgA+ cells in these tissues bound SC intracellularly (7) and, therefore, probably produce polymeric IgA (6). In contrast, Radl et al. (8) observed that only 7.3 ± 5.1% of IgA plasma cells in normal human bone marrow are capable of SC binding. These results indicate that tissues associated with mucosal surfaces produce predominantly polymeric forms, whereas bone marrow rich in IgA plasma cells (19) produces primarily IgA monomers. Results of the present investigation are supported by our previous observations that indicated that after culturing normal PBL for 7 d with PWM, 21.8–71.4% of IgA-producing cells bound SC (13). Now we report that ~60–70% of the IgA released by these cells occurs as a polymer. However, it is not clear whether an IgA-producing plasma cell has a potential to produce either polymer, monomer, or both forms concurrently or sequentially. If one accepts the ability of some IgA plasma cells to bind SC as a criterion of polymer production, it is obvious

that there are numerous cells, especially in the bone marrow (5), that do not bind SC and are, therefore, engaged in the production of IgA monomers. Low levels of polymeric IgA in human sera may be explained by their prompt and effective removal from the circulation and subsequent selective transport into external secretions by secretory glands and liver (20–22).

In summary, a large proportion of peripheral blood B-lymphocytes, with a potential to differentiate into IgA plasma cells, produce predominantly polymeric IgA and display an IgA subclass distribution typical of external secretions. These findings suggest that precursors of IgA-synthesizing cells found in the blood may be destined to populate the subepithelial surfaces of mucosal tissues. This has been predicted by several investigators who proposed a specialized migratory cycle for IgA precursor cells (9–12).

Summary

Human peripheral blood lymphocytes (PBL) were cultured for various time periods (up to 8 d) in the presence of pokeweed mitogen (PWM), lipopolysaccharide, or Epstein-Barr virus. Cell-free supernates were fractionated on a standardized Ultrogel AcA 22 column and the proportion of polymeric and monomeric IgA was determined by radioimmunoassay. The results demonstrate that PBL stimulated with these mitogens produce IgM and IgG with molecular characteristics identical to those found in serum, but that the IgA produced is predominantly of the polymeric type. To prove that this IgA represented disulfide bond-linked polymers rather than aggregated monomers, we have demonstrated that the high molecular weight IgA (*a*) maintains its polymeric form upon treatment with acidic buffers, (*b*) contains J chain, a glycoprotein associated only with polymeric immunoglobulins, and (*c*) dissociates to the monomeric form upon reduction of disulfide bonds. After 1 wk in culture, ~60% of the PWM-stimulated cells that contained IgA were positive for IgA2, whereas 40% were IgA1 positive as determined by immunofluorescence. Therefore, peripheral blood contains a population of lymphocytes with the potential to display, after appropriate stimulation and differentiation, characteristics similar to IgA cells found in external secretory tissues. The demonstration of the presence of such cells in the peripheral circulation suggests that these cells are precursors of IgA-producing plasma cells with the potential to populate mucosal tissues.

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References

1. Heremans, J. F. 1974. Immunoglobulin A. *In* The Antigens. M. Sela, editor. Vol. 2. Academic Press, Inc., New York. 365.
2. Vaerman, J.-P., J. F. Heremans, and C.-B. Laurell. 1968. Distribution of α-chain subclasses in normal and pathological IgA-globulins. *Immunology.* **14**:425.
3. Grey, H. M., C. A. Abel, J. Yount, and H. G. Kunkel. 1968. A subclass of human γA-globulins (γA2) which lacks the disulfide bonds linking heavy and light chains. *J. Exp. Med.* **128**:1223.

4. Skvaril, F., and A. Morell. 1974. Distribution of IgA subclasses in sera and bone marrow plasma cells of 21 normal individuals. *Adv. Exp. Med. Biol.* **45**:433.
5. Mach, J. P. 1970. *In vitro* combination of human and bovine free secretory component with IgA of various species. *Nature (Lond.).* **228**:1278.
6. Radl, J., F. Klein, P. van den Berg, A. M. de Bruyn, and W. Hijmans. 1971. Binding of secretory piece to polymeric IgA and IgM paraproteins *in vitro*. *Immunology.* **20**:843.
7. Brandtzaeg, P. 1973. Two types of IgA immunocytes in man. *Nat. New Biol.* **243**:142.
8. Radl, J., H. R. E. Schuit, J. Mestecky, and W. Hijmans. 1974. The origin of monomeric and polymeric forms of IgA in man. *Adv. Exp. Med. Biol.* **45**:57.
9. Lamm, M. E. 1976. Cellular aspects of immunoglobulin A. *Adv. Immunol.* **22**:223.
10. Cebra, J. J., P. J. Gearhart, R. Kamat, S. M. Robertson, and J. Tseng. 1977. Origin and differentiation of lymphocytes involved in the secretory IgA response. *Cold Spring Harbor Symp. Quant. Biol.* **41**:201.
11. Mestecky, J., J. R. McGhee, S. M. Michalek, R. R. Arnold, S. S. Crago, and J. L. Babb. 1978. Concept of the local and common mucosal immune response. *Adv. Exp. Med. Biol.* **107**:185.
12. Bienenstock, J., M. McDermott, and D. Befus. 1979. A common mucosal immune system. *In* Immunology of Breast Milk. P. L. Ogra and D. H. Dayton, editors. Raven Press, New York. 91.
13. Crago, S. S., and J. Mestecky. 1979. Secretory component: interactions with intracellular and surface immunoglobulins of human lymphoid cells. *J. Immunol.* **122**:906.
14. Conley, M. E., J. F. Kearney, A. R. Lawton, and M. D. Cooper. Differentiation of human B cells expressing the IgA subclasses as demonstrated by monoclonal hybridoma antibodies. *J. Immunol.* In press.
15. Hunter, W. M. 1978. Radioimmunoassay. *In* Handbook of Experimental Immunology. D. M. Weir, editor. Blackwell Scientific Publications Ltd., Oxford, England. 14.1.
16. Schrohenloher, R. E., H. G. Kunkel, and T. B. Tomasi. 1964. Activity of dissociated and reassociated 19S anti γ globulins. *J. Exp. Med.* **120**:1215.
17. André, C., F. André, and M. C. Fargier. 1978. Distribution of IgA1 and IgA2 plasma cells in various normal human tissues and in the jejunum of plasma IgA-deficient patients. *Clin. Exp. Immunol.* **33**:327.
18. Tomasi, T. B., Jr., D. Bull, D. Tourville, M. Montes, and A. Yurchak. 1971. Distribution and synthesis of human secretory components. *In* The Secretory Immunologic System. D. H. Dayton, Jr., P. S. Small, Jr., R. M. Chanock, H. E. Kaufman, and T. B. Tomasi, Jr., editors. U. S. Government Printing Office, Washington, D. C. 41.
19. Hijmans, W., H. R. E. Schuit, and E. Hulsing-Hesselink. 1971. An immunofluorescent study on intracellular immunoglobulins in human bone marrow cells. *Ann. N. Y. Acad. Sci.* **177**:290.
20. Virella, G., P. C. Montgomery, and I. M. Lemaître-Coelho. 1978. Transport of oligomeric IgA of systemic origin into external secretions. *Adv. Exp. Med. Biol.* **107**:241.
21. Orlans, E., J. Peppard, J. Reynolds, and J. Hall. 1978. Rapid active transport of immunoglobulin A from blood to bile. *J. Exp. Med.* **147**:588.
22. Lemaître-Coelho, I., G. D. F. Jackson, and J.-P. Vaerman. 1978. High levels of secretory IgA and free secretory component in the serum of rats with bile duct obstruction. *J. Exp. Med.* **147**:934.