

Brief Definitive Reports

MONOCLONAL PRODUCTION OF BOTH IgM AND IgG1 ANTIHAPTEN ANTIBODY*

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Investigations on the clonal nature of antibody formation have demonstrated the expression of allelic exclusion by lymphoid cells (1), as well as the production of a monospecific, restricted population of antibody molecules by the clonal progeny of a single antibody-forming precursor cell (B cell) (2, 3).¹ Recent studies suggest, however, that a single clone may synthesize antibody molecules of the same specificity which differ in heavy chain class. Thus, idiotypic determinants, which are considered to be a function of the antibody combining site and therefore a variable region marker (4), have been shown to be shared among the IgM and IgG anti-Salmonella antibodies produced by individual rabbits (5). Furthermore, IgG and IgM myeloma proteins derived from a single individual have been shown to share idiotypic determinants (6, 7) while structural studies indicate that these proteins have identical light chains and heavy chain variable region subgroups (7-9). These and other findings (10, 11) are consistent with the hypothesis that the primary amino acid sequence of a single light or heavy chain is encoded by two genes and that the synthesis of the polypeptide chain results from a mechanism involving translocation (11, 12).

It is conceivable that a lymphoid cell might "switch" from IgM to IgG antibody production while maintaining the same or similar antibody specificity. That such a switch mechanism is operative has been implied by several findings. Pernis et al. (13, 14) have demonstrated that lymphocytes with IgM molecules on the cell membrane possess, in their cytoplasm, IgG molecules with similar allotypic determinants. Several investigators have shown that treatment of lymphoid cells with anti- μ chain antiserum inhibits the production of IgG antibody-forming cells in response to antigenic stimulation (15-17). Finally, Nossal et al. (18, 19) noted that microdroplets containing single antibody-forming cells expressed both IgM and IgG antibodies.

The studies presented here were conducted to determine whether the clonal progeny of a single stimulated precursor cell produced antibody of more than one heavy chain class in response to a defined, haptenic determinant. Analysis of antibodies produced by splenic foci derived from the adoptive transfer of limiting numbers of clonal precursor cells indicated that foci producing both

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IgM and IgG1 antihapten antibodies occurred at a significantly greater than random frequency.

Materials and Methods

Antigens.—The preparation of *Limulus polyphemus* hemocyanin (Hy) and 2,4-dinitrophenylated hemocyanin (DNP-Hy) has been described previously (3). DNP-Hy contained 10 mol of DNP per 100,000 g of Hy.

Animals.—8–10-wk old Balb/c mice (Carworth Div. Becton, Dickinson, and Co., New York) were immunized by an intraperitoneal injection of 0.1 mg of DNP-Hy in complete Freund's adjuvant (CFA) and used 4–6 mo later as secondary spleen cell donors. Balb/c recipients for cell transfer were carrier-primed 4–8 wk before use by an intraperitoneal injection of 0.1 mg of Hy in CFA (3). Nonimmune Balb/c mice were used as primary spleen cell donors at 8–10 wk of age.

Cell Transfer.—Donor spleen cell suspensions were prepared in Dulbecco's modified Eagle's medium using a Teflon tissue homogenizer (3). Carrier-primed recipients received intravenously $0.5\text{--}2.0 \times 10^6$ viable spleen cells 6 h after 1,000 r total body irradiation from a cesium source.

Spleen Fragment Cultures.—Recipient spleen fragments were cultured by methods described previously (3), and stimulated with 1 $\mu\text{g}/\text{ml}$ of DNP-Hy, 10^{-7} M for DNP. Culture fluids were collected every 2–3 days thereafter, and analyzed for antibody by the radioimmunoassay 7, 11, 13, and 15 days after in vitro stimulation.

Radioimmunoassay.—The radioimmunoassay of culture fluids for anti-DNP antibody and anti-Hy antibody, and the preparation of iodinated, purified rabbit antimouse Fab fragment antibody has been described previously (20). MOPC 104E myeloma protein (μ , λ) was purified from tumor tissue and coupled to bromoacetyl cellulose by methods described previously.¹ Lambda protein was purified from the urine of RPC-20 myeloma tumor-bearing mice by the method of Potter (21) and coupled to cyanogen bromide activated Sepharose (Pharmacia Fine Chemicals, Piscataway, N. J.). MOPC 31c (γ 1, K) myeloma protein was purified from tumor tissue by methods described previously,¹ and coupled to Sepharose.

Antisera specific for different classes of mouse immunoglobulin heavy chains, produced in goats by immunization with purified myeloma proteins, and adsorbed to remove cross-reactivity with light and heavy chains of other myeloma proteins, were obtained from Meloy Laboratories, Inc., Springfield, Va. Goat antimouse μ -chain antibody was purified from the Meloy anti- μ antiserum by adsorption on 104E myeloma protein coupled immunoadsorbent and subsequent elution with 0.1 N acetic acid.¹ Iodinated goat antimouse μ -chain antibody was passed through a lambda-protein coupled Sepharose immunoadsorbent. Goat antimouse γ 1-chain antibody was purified from the Meloy anti- γ 1 antiserum by adsorption on MOPC 31C (IgG1) protein coupled immunoadsorbent and elution with 0.008 M glycylglycine HCl, 0.3 M for NaCl, pH 2.25. This preparation was iodinated as described previously (20).

The purified preparations of goat antimouse μ -chain antibody and goat antimouse γ 1-chain antibody were shown to have no demonstrable cross-reactivity for other heavy chain determinants or for light chains. This was established by determining that neither iodinated purified antibody preparation bound to mouse Fab fragment coupled to Sepharose. Furthermore, iodinated goat antimouse μ -chain antibody did not bind to IgG molecules purified from serum by DEAE-cellulose chromatography and coupled to bromoacetyl cellulose.¹ Antimouse γ 1-chain antibody showed no binding to IgM molecules purified from serum by G200 Sephadex gel filtration and coupled to bromoacetyl cellulose.

RESULTS

Culture fluids obtained 11 and 13 days after in vitro stimulation of spleen fragments with DNP-Hy were analyzed by the radioimmunoassay, using

specifically purified antibodies against mouse heavy chain determinants (μ and $\gamma 1$), and mouse light chain determinant (Fab fragment). Table I presents the results of such an analysis. 58% of the primary foci produced IgG1 anti-DNP antibody; 32% produced IgM anti-DNP antibody; and 16% produced both IgM and IgG1 anti-DNP antibody. While 82% of the secondary foci produced IgG1 anti-DNP antibody, no secondary foci were detected which produced solely IgM anti-DNP antibody. 14% of the secondary foci produced both IgM and IgG1 antihapten antibody. The frequencies of IgM and IgG1 antihapten antibody producing primary and secondary foci remained essentially the same when culture fluids were analyzed as early as 7 days after stimulation.

TABLE I
Analysis of Immunoglobulin Class of Antibody Produced by Splenic Foci

Anti-DNP antibody detected by	Primary foci		Secondary foci*	
	Foci	Total foci*	Foci	Total foci
	<i>no.</i>	%	<i>no.</i>	%
Anti-Fab	10	26	7	18
Anti-Fab or anti- $\gamma 1$	14	36	25	68
Anti-Fab or anti- μ	5	13	0	0
Anti-Fab or anti- $\gamma 1$ or anti- μ	5	13	5	14
Anti- $\gamma 1$	2	6	0	0
Anti- μ	1	3	0	0
Anti- $\gamma 1$ or anti- μ	1	3	0	0
Total foci detected	38		37	
Total cells injected	14×10^6		5×10^6	
Total fragments analyzed	336		480	

* All fragments stimulated with 10^{-7} M DNP determinant concentration on DNP-Hy.

Table II presents the frequencies of primary and secondary foci producing more than one heavy chain class of anti-DNP antibody and the frequencies which would be predicted by a random distribution. It is apparent that the number of foci obtained which produced both IgM and IgG1 antihapten antibody is considerably higher than that predicted by random occurrence. In contrast, the number of primary foci producing both anti-DNP antibody and anti-Hy antibody is close to that predicted by a random distribution.

DISCUSSION

Previous reports from this laboratory have demonstrated that the number of splenic foci detected after transfer of limiting numbers of nonimmune or immune spleen cells to carrier-primed recipients is linearly related to the number of cells injected, and that the antihapten antibodies produced by these foci are restricted by the criteria of isoelectric spectra and hapten-binding properties (3).¹ These findings suggest that such primary and secondary splenic foci are the result of antibody production by the clonal progeny of a single stimulated B cell. This is in contrast to studies using erythrocyte antigens, where splenic

TABLE II
 (A) *Frequency of Splenic Foci Releasing IgM and IgG1 Anti-DNP Antibody**

Anti-DNP antibody heavy chain class	Number of	
	Primary foci	Secondary foci
$\gamma 1$	22	30
μ	12	5
$\gamma 1$ and μ	6 (0.8)‡	5 (0.3)‡
Total fragments analyzed	336	480

(B) *Frequency of Splenic Foci Releasing Anti-DNP and Anti-Hy Antibody**

Specificity of antibody released per focus	Number of primary foci
DNP	102
Hy	19
DNP and Hy	4 (2.8)‡
Total fragments analyzed	570

* All fragments stimulated with 10^{-7} M DNP determinant concentration on 1 $\mu\text{g}/\text{ml}$ DNP-Hy.

‡ Number of fragments predicted for random occurrence of two simultaneous, independent events.

foci produced heterogeneous antibodies under conditions of B cell excess (22). In the studies reported here, the limiting cell for focus formation is the antibody-forming precursor cell. This is reinforced by the finding that after transfer of limiting numbers of nonimmune spleen cells to carrier-primed recipients, the frequency of primary foci producing both antihapten antibody and anticarrier antibody is not significantly higher than that predicted by a random event.

An analysis of the heavy chain class of the antihapten antibody produced by these splenic foci indicated that 16% of the primary foci and 14% of the secondary foci produced both IgM and IgG1 anti-DNP antibody. Since the observed frequency of these foci is higher than that predicted by a random distribution, it is unlikely that the IgM and IgG1 antibody produced by these foci is the result of two different hapten-specific B cells residing in the same fragment. It would thus appear that the clonal progeny of a single precursor cell can produce IgM and IgG1 antibody which is specific for a single determinant. These studies do not indicate, however, whether the "monofocal" production of IgM and IgG1 antihapten antibody is the result of one or more antibody-forming cells within the focus switching from IgM to IgG1 antibody production, or whether this is the result of IgM antibody production by some cells of a clone while others produce IgG1 antibody. It should be noted that the production of IgM and IgG1 anti-DNP antibody by a single focus does not constitute defini-

tive evidence for the sharing of heavy chain variable region sequences by these antibodies. Such evidence would require analysis of the variable regions of these antibodies.

Less than 20% of the primary or secondary foci which produced IgG1 antibody also produced IgM antibody, even as early as 7 days after stimulation. This finding implies that the production of IgM antibody is not requisite for the production of IgG1 antibody by the clonal progeny of a single precursor cell. While both primary and secondary foci produced predominantly IgG1 anti-DNP antibody, primary but not secondary foci were detected which produced solely IgM antibody. Those secondary foci which produced IgM antibody also produced IgG1 antibody. The absence of secondary foci making only IgM antibody may denote a qualitative difference between primary and secondary DNP-specific precursor cells, and their clonal progeny.

SUMMARY

The anti-DNP antibodies produced by primary and secondary splenic foci were analyzed for heavy chain class by a radioimmunoassay, using iodinated, purified goat antimouse μ -chain antibody and goat antimouse γ 1 chain antibody. The frequency of primary and secondary foci producing both IgM and IgG1 anti-DNP antibody (16% and 14%, respectively) was considerably higher than that which would be predicted by a random distribution. It would thus appear that IgM and IgG1 antibody can be made by the clonal progeny of a single precursor cell.

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