Brief Definitive Report

IMMUNE NETWORKS

Frequencies of Antibody- and Idiotype-producing B Cell Clones in Various Steady States*

By ROSA R. BERNABÉ, ANTONIO COUTINHO,‡ CARLOS MARTINEZ-A., and PIERRE-ANDRÉ CAZENAVE

From the Basel Institute for Immunology, Basel, Switzerland; the Department of Immunology, Umeå University, Umeå, Sweden; and the Laboratory of Analytic Immunochemistry, Pasteur Institute, Paris

The network theory of the immune system (1) can be reduced to the interactions between soluble and cell-bound idiotypes and combining sites, independently of the functional properties of the lymphocytes bearing those elements. After its original exposition, however, much attention has been drawn to the functionally opposing consequences of the activity mediated by distinct classes of lymphocytes. The "plus-minus" general network interactions have thus been progressively substituted by "mini-networks" of suppressor and helper T cells, often disconnected from the overall regulatory influences maintaining steady states in a complete, and therefore circular, repertoire.

It appeared important, therefore, to establish a primary, quantitative description of the immune elements in various steady states to provide the basis for a description of immune networks. This, on the other hand, would perhaps contribute to resolving the present problems of predicting pathways in network interactions and the consequences of specific manipulations. We have initiated these attempts by determining B cell precursor frequencies in quantitative assays (2, 3), under conditions that limit only for the clonal precursor itself. This was done in a well-characterized system prototype of network regulation (4–6) using monoclonal idiotypes and anti-idiotypes (7).

Materials and Methods

Mice. BALB/c mice were bred at the Pasteur Institute, Paris, or obtained from the Institute for Biomedical Research, Füllinsdorf, Switzerland, and used between 2 and 8 mo of age.

Immunizations. Antigen immunity was induced by priming with either trinitrophenyl (TNP)-Ficoll or TNP-ovalbumin as previously described (6). Ab_2 mice were prepared by immunization with MOPC460 myeloma protein, copolymerized with keyhole limpet hemocyanin by means of glutaraldehyde, and emulsified in complete Freund's adjuvant as described (8). Ab_3 mice were those immunized with either monoclonal F6(51) (7) or conventional anti-idiotypic antibodies, according to the same protocol.

Antibody Determinations. These were done in a hemagglutination (HA) assay using either TNP-substituted sheep erythrocytes (SRBC) or erythrocytes coupled to the idiotype (MOPC460

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‡ To whom correspondence should be addressed at the Department of Immunology, Umeå University, 90185 Umeå, Sweden.

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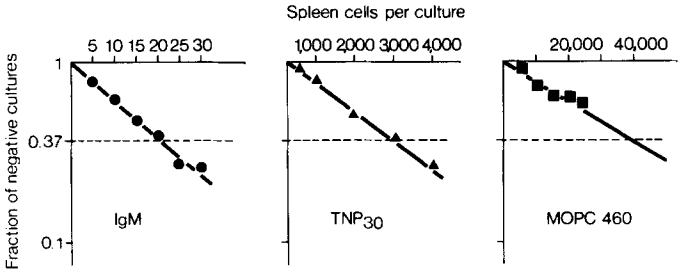

FIG. 1. The indicated numbers of spleen cells from normal BALB/c mice were cultured in the presence of LPS in groups of 48, replicates and assayed on day 5 for IgM-secreting cells with protein-A-SRBC (●), as well as for direct anti-TNP PFC using TNP_{30}-SRBC (▲). The presence of the MOPC460 idiotype was assayed by RHA, in the supernates after 10 d of culture (■).

myeloma protein). Quantitation of idiotype was done in a reverse HA (RHA) assay, by coupling to SRBC, purified F6(51) anti-idiotypic antibodies. The sensitivity of the assay was evaluated by using the purified myeloma protein MOPC460; agglutination was obtained with idiotype concentrations from 10 μg to 500 picog/ml. No agglutination was observed when irrelevant myeloma proteins (TEPC15 and MOPC315) were used. Radioimmunoassays (6) were also used in some experiments, but the sensitivity and specificity of the RHA assay has proved superior to radioimmunoassays and was therefore used throughout this study.

Frequency Determinations. These were performed as previously described (2, 3), by diluting spleen cells in 0.2-ml cultures containing 6×10^5 rat filler thymus cells and optimal concentrations of lipopolysaccharide (LPS) from *Escherichia coli* (055:B5; Difco Laboratories, Detroit, Mich.), in medium containing 5×10^{-5} 2-mercaptoethanol and 10–20% of fetal calf serum. Cultures were assayed on day 5 for IgM-secreting cells in the protein A-plaque assay (9) and for direct anti-TNP plaque-forming cells (PFC) using densely substituted (TNP_{30}) erythrocytes. The scoring of idiotype-producing cultures was done in the supernates after 10–12 d of culture by RHA. In each experiment, at least five different cell concentrations were cultured in 48 replicates, for each of the specificities detected.

Results and Discussion

The limiting dilution culture and assay systems used here allow for the calculation of precursor cell frequencies. Thus, as shown in Fig. 1, the plots of the numbers of cells per culture vs. the log of the fraction of negative cultures are straight lines intercepting the origin, demonstrating that only the clonal precursors themselves are limiting in the assays (10). This excludes influences of either helper or suppressive cells that might be postulated in the system and, therefore, these experiments provide direct information on the B cell compartment at the time the animals were killed. The specific frequency determinations among all spleen cells must be related to internal controls for the plating efficiency in the system, namely for the total frequency of immunoglobulin-secreting clones, determined in the same cell suspensions in each experiment. This provides the absolute frequencies of a given specificity among mitogen-reactive cells. As shown in Fig. 1, in normal BALB/c mice, roughly 1:2,000–1:3,000 immunoglobulin-secreting B cell clones produce an immunoglobulin bearing the MOPC460 idiotype recognized by the monoclonal antibody used here. This frequency compares well with that of A5A idiotype-producing cells previously determined in this system (11). The frequencies of anti-TNP-secreting precursors are also comparable to those previously described (3).

The analysis of the same frequencies in BALB/c mice manipulated to various immune steady states are shown in Table I. Mice immunized with the antigen TNP

TABLE I

*Frequencies of LPS-reactive B Cells Producing Anti-TNP Antibodies Bearing MOPC460 Idiotype in Various Immune Steady States**

	Frequency per total spleen cells	Frequency of specific B cells within the LPS-reactive population
Normal BALB/c		
IgM	1/17	
TNP	1/3,000	1/176
MOPC460	1/40,000	1/2,500
Ab_1 BALB/c		
IgM	1/15	
TNP	1/1,200	1/80
MOPC460	1/12,000	1/800
Ab_2 BALB/c		
IgM	1/15	
TNP	1/3,000	1/200
MOPC460	1/200,000	1/14,000
Ab_3 BALB/c		
IgM	1/40	
TNP	1/3,500	1/87
MOPC460	1/15,000	1/380

* See legend to Fig. 1.

(Ab_1) show a moderate increase in the frequencies of both antibody- and idiotype-secreting clones (two- to threefold the control). Mice immunized with the idiotype (Ab_2), on the other hand, show a selective suppression of idiotype-secreting clones (below detectable levels), while maintaining normal frequencies of antibody-secreting clones. Finally, mice immunized with the anti-idiotype (Ab_3) display a markedly increased frequency of idiotype-producing clones (5–10 times higher than controls in different experiments) and a comparatively smaller increase in antibody-forming precursors. These results are in general agreement with the immune responses previously determined in these mice. Thus, Ab_2 mice respond normally to TNP but fail to include the relevant idiotype in that response, whereas Ab_3 mice mount augmented antibody responses in which the MOPC460 idiotype component is disproportionately represented (12, 13). These response patterns have previously been attributed to the activity of regulatory T cells (14). As shown here, however, they are well correlated with B cell precursor frequencies. These, in turn, appear to be modulated by regulatory T cells (14), but it is clear that these regulatory processes occur before the introduction of antigen, conventionally administered to test the immune status, but avoided here in our protocol of frequency determinations. This is why we feel that our results reflect established immune states that are stable for at least several months after the manipulations used to establish them.

It must be pointed out that our results only concern B cells composing the LPS-reactive subset. It may be expected, therefore, that the perturbation of specific frequencies in these various immune states before the introduction of antigen are even more marked in other functionally defined B cell populations. Results shown in Table II could support this possibility. Thus, sera collected from the same mice in the spleen cells of which these frequencies were determined show differences in the titers of circulating idiotype that are much greater than the corresponding difference in precursor frequencies. This emphasizes the lack of an assay for frequency determina-

TABLE II

Titers of Circulating Anti-TNP Antibodies and MOPC460 Idiotype in Various Steady States

Sera from	Antibody titers	
	Anti-TNP*	MOPC460 idiotype‡
Normal BALB/c	1/2	<1/2
Ab_1 BALB/c	1/64–1/356	1/16
Ab_2 BALB/c	1/8–1/16	<1/2
Ab_3 BALB/c	1/8–1/16	>1/4,096

* As detected by HA using TNP-SRBC.
‡ As detected by RHA using F6(51)-coupled SRBC.

tions among other B cell subsets (thymus-dependent B cells in particular) with the same quantitative requirements and internal control for plating efficiencies as those available for thymus-independent mitogens.

Our results demonstrate the ability of the normal immune system to modulate its steady state by self-recognition of its own elements. It will be important to determine whether the modifications in B cell precursor frequencies result from selective mechanisms operating at the level of bone marrow precursors and regulating their output to the competent cell pool, or whether they result from the expansion or suppression of mature, peripheral clones. In view of the very rapid turnover of LPS-reactive cells in the spleen (A. Freitas and A. Coutinho, manuscript submitted for publication), the first alternative would appear more likely at the present time.

Summary

In limiting dilution analysis, the absolute frequencies of lipopolysaccharide-reactive B cell precursors producing anti-trinitrophenyl antibodies or the MOPC460 idiotype were studied in BALB/c mice, either normal, or immunized with antigen (Ab_1), the idiotype (Ab_2), or a monoclonal anti-idiotype antibody (Ab_3). Anti-idiotype immunity results in the suppression of the B cell precursors for the relevant idiotype, and anti-(anti-idiotype) immunity leads to a 10-fold increase in precursor B cell frequencies, with a comparatively lower increase in antibody-producing precursors. The findings can only be explained by variations in the composition of the B cell compartment in the various immune states.

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References

1. Jerne, N. K. 1974. Toward a network theory of the immune system. *Ann. Immunol. (Paris).* **125:**373.
2. Andersson, J., A. Coutinho, W. Lernhardt, and F. Melchers. 1977. Clonal growth and maturation to immunoglobulin secretion "in vitro" of every growth-inducible B-lymphocyte. *Cell.* **10:**27.
3. Andersson, J., A. Coutinho, and F. Melchers. 1977. Frequencies of mitogen-reactive B cells in the mouse. II. Frequencies of B cells producing antibodies which lyse sheep or horse

erythrocytes and trinitrophenylated or nitroiodophenylated sheep erythrocytes. *J. Exp. Med.* **145:**1520.

4. Cazenave, P.-A. 1977. Idiotypic-anti-idiotypic regulation of antibody synthesis in rabbits. *Proc. Natl. Acad. Sci. U. S. A.* **74:**5122.
5. Urbain, J., M. Wikler, J. D. Franssen, and C. Collignon. 1977. Idiotypic regulation of the immune system by the induction of antibodies against anti-idiotypic antibodies. *Proc. Natl. Acad. Sci. U. S. A.* **74:**5126.
6. Le Guern, C., F. Ben Aissa, D. Juy, B. Mariamé, G. Buttin, and P.-A. Cazenave. 1979. Expression and induction of MOPC-460 idiotypes in different strains of mice. *Ann. Immunol. (Paris).* **130:**293.
7. Buttin, G., C. Le Guern, L. Phalente, E. C. C. Lin, L. Medrano, and P.-A. Cazenave. 1978. Production of hybrid lines secreting monoclonal anti-idiotypic antibodies by cell fusion on membrane filters. *In* Current Topics in Microbiology and Immunology. F. Melchers, M. Potter, and N. Warmer, editors. Springer-Verlag, Berlin, Heidelberg. **81:**27.
8. Sakato, N., and H. N. Eisen. 1975. Antibodies to idiotypes of isologous immunoglobulins. *J. Exp. Med.* **141:**1411.
9. Gronowicz, E., A. Coutinho, and F. Melchers. 1976. A plaque assay for all cells secreting Ig of a given type or class. *Eur. J. Immunol.* **6:**588.
10. Lefkovits, I., and H. Waldmann. 1979. Limiting Dilution Analysis of Cells in the Immune System. Cambridge University Press, Cambridge, England.
11. Eichmann, K., A. Coutinho, and F. Melchers. 1977. Absolute frequencies of lipopolysaccharide-reactive B cells producing A5A idiotype in unprimed, streptococcal A carbohydrate-primed, anti-A5A idiotype-sensitized and anti-A5A idiotype-suppressed A/J mice. *J. Exp. Med.* **146:**1436.
12. Bona, C., P.-A. Cazenave, and W. E. Paul. 1979. Regulation of anti-TNP response by anti-idiotypic and anti(anti-idiotypic) antibodies. *Ann. Immunol. (Paris).* **130:**303.
13. Bona, C., R. Hooghe, P.-A. Cazenave, C. Le Geurn, and W. E. Paul. 1979. Cellular basis of regulation of expression of idiotype. II. Immunity to anti-MOPC-460 idiotype antibodies increases the level of anti-trinitrophenyl antibodies bearing 460 idiotypes. *J. Exp. Med.* **149:** 815.
14. Bona, C., and W. E. Paul. 1979. Cellular basis of regulation of expression of idiotype. I. T-suppressor cells specific for MOPC 460 idiotype regulate the expression of cells secreting anti-TNP antibodies bearing 460 idiotype. *J. Exp. Med.* **149:**592.