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 conse-
quences 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, Fullinsdorf, 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). Ab₂ 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). Ab₀ 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.
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 ng to 500 picogram/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 X 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 X 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 (TNP30) 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
(Ab1) show a moderate increase in the frequencies of both antibody- and idiotype-secreting clones (two- to threefold the control). Mice immunized with the idiotype (Ab2), 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 (Ab3) 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, Ab2 mice respond normally to TNP but fail to include the relevant idiotype in that response, whereas Ab3 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

<table>
<thead>
<tr>
<th>Sera from</th>
<th>Anti-TNP**</th>
<th>MOPC460 idiotype‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal BALB/c</td>
<td>1/2</td>
<td>&lt;1/2</td>
</tr>
<tr>
<td>Ab BALB/c</td>
<td>1/64-1/356</td>
<td>1/16</td>
</tr>
<tr>
<td>Ab2 BALB/c</td>
<td>1/8-1/16</td>
<td>&lt;1/2</td>
</tr>
<tr>
<td>Ab3 BALB/c</td>
<td>1/8-1/16</td>
<td>&gt;1/4,096</td>
</tr>
</tbody>
</table>

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

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), the idiotype (Ab2), or a monoclonal anti-idiotype antibody (Ab3). 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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