VARIATION IN ACCESSIBLE CELL SURFACE IMMUNOGLOBULIN AMONG ANTIBODY-FORMING CELLS*

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(Received for publication 24 March 1972)

There is now good agreement that immunoglobulin receptors on the surface of bone marrow-derived (B) lymphocytes play an important role in the activation of such lymphocytes by antigen (1–3). Antibody-secreting cells are derived through mitosis and differentiation of precursor B cells, and the process of clonal expansion can involve numerous sequential mitoses (4, 5). It thus becomes of interest to ask whether antigen-activated lymphocytes retain surface Ig receptors, and, if so, whether the density of receptors per cell varies with progressive maturation of the clone. Diminution or disappearance of receptor would obviously reduce or abolish the regulatory influence which extracellular antigen could exert on the cell concerned. A number of studies, directly or indirectly related to this question, have given conflicting results.

Early work on the phenomenon of immunocytoadherence (6, 7) was predicated on the hypothesis that antibody-forming cells had a coating of their secretory product firmly attached to the cell surface, and substantial evidence for this view has since accumulated (8, 9). On the other hand, work using membrane immunofluorescence has suggested that plasma cells, known to be fully differentiated antibody-secreting cells, cannot be shown to possess surface Ig by methods which readily demonstrate Ig receptors on B lymphocytes (10). Reports that cells of most plasma cell tumors lack surface Ig have also appeared (11, 12).

We have recently studied thymus (T) and B lymphocyte receptors (13) and problems of their metabolic turnover (14) by quantitative radioautographic techniques in which specific radiolabeled antiglobulins were attached to living lymphocytes at 0°C. The present report deals with an application of this approach, combined with the technique of micromanipulation, to the question of Ig density on the surface of single plaque-forming cells (PFC). While this work was in progress, two highly relevant papers were published (12, 15). Takahashi et al. (12) subjected PFC to the action of antoglobulin sera and complement.

* This work was supported by grants from the National Health and Medical Research Council and the Australian Research Grants Committee, Canberra, Australia, and from the US Public Health Service (AI-O-3958). This is publication No. 1706 from the Walter and Eliza Hall Institute.

1 Abbreviations used in this paper: B, bone marrow; PFC, plaque-forming cells; SRBC, sheep erythrocytes; T, thymus.
They found that 60% of IgM-forming PFC but no IgG-forming PFC displayed detectable surface Ig. McConnell, using an elegant combined rosette-plaque method (15), studied IgG-forming PFC in a secondary response. He did find evidence for surface receptors on some IgG-formers, the proportion of positive cells being higher at early times in the secondary response. Our findings reported here are in broad agreement with McConnell, and add more quantitative data.

Materials and Methods

Animals.—Male CBA/H/Wehi mice aged 10-12 wk at first immunization were used throughout.

Immunization.—Mice studied for the primary response received a single intraperitoneal injection of $10^9$ sheep red blood cells (SRBC). Mice used for the secondary response received $10^6$ SRBC as a primary dose and $10^9$ SRBC 3-4 wk later.

Labeling of Lymphocytes with Antiglobulin.—Spleen cells were used throughout. They were reacted for 30 min at 0°C with a 125I-labeled 7S globulin fraction from a strong, polyclonal (predominantly anti-$\kappa$ and anti-$\lambda$) rabbit anti-mouse globulin serum, R19, at a concentration of 1 $\mu$g/ml, specific activity 5-10 $\mu$Ci/$\mu$g. Details of these techniques have been previously published (13, 14).

Micromanipulation and Plaque Techniques.—Our aim was to study the amount of Ig at the surface of the PFC at the moment of killing of the animal. Previous work had shown that incubation at 37°C caused rapid shedding of attached antireceptor (14). It was thus important to minimize the length of incubation of labeled cells required for plaque revelation. This consideration favored the liquid monolayer plaque technique (16) where plaques appear within 5-15 min, over agar methods where sequential incubations may take several hours. Accordingly, cells labeled in the cold were placed in microdroplets under plaque-revealing conditions, and within minutes of first plaque appearance were micromanipulated singly onto premarked circles on gelatin-coated slides for radioautography. After fixation, dipping, exposure, and development the cells were stained with Giemsa. Details of all the relevant methods have been previously published (17, 4). Both direct (largely IgM) and indirect (largely IgG) PFC were studied.

Quantitative Radioautography.—Grain counts were performed on the single PFC and on non-PFC handled identically. Variations in specific activity of different batches of antiglobulin-125I, in time lag between micromanipulation and dipping of radioautographs, and in exposure period occurred. Therefore, actual grain counts were normalized to standard conditions of 5 $\mu$Ci/$\mu$g of radioactivity and 3 days' exposure.

RESULTS

A total of 164 single PFC in the primary response and 94 single PFC in the secondary response from 18 experiments were studied for intensity of binding of antiglobulin. The full grain count data are presented in Fig. 1. It is immediately apparent that great heterogeneity in grain counts was encountered. When direct and enhanced PFC from a particular day point were compared, no significant difference in grain count distribution was noted. However, when labeling was considered in relation to time after immunization, a trend became apparent. This is illustrated in Table I. In general, there were more unlabeled cells and fewer heavily labeled cells at later stages of the immune
Fig. 1. Scatter diagram of binding of anti-Ig to single PFC at 0°C in vitro. Each point represents one cell. Grain counts were normalized as described in the text.

### TABLE I

Relation between Time after Immunization and Intensity of Binding of Anti-Ig to PFC

| Response | Time after immunization | Number of single PFC examined | Per cent of cells |
|----------|--------------------------|------------------------------|-------------------|
|          |                          |                              | Unlabeled* | Lightly labeled† | Heavily labeled§ |
| Primary  |                          |                              |            |                 |                  |
| day      |                          |                              |            |                 |                  |
| 3        | 51                       | 16                           | 23         | 61               |
| 4        | 26                       | 8                            | 19         | 73               |
| 5        | 14                       | 0                            | 29         | 71               |
| 7        | 32                       | 28                           | 47         | 25               |
| 9 to 10  | 36                       | 56                           | 22         | 22               |
| 21       | 5                        | 100                          | 0          | 0                |
| Secondary|                          |                              |            |                 |                  |
| 3        | 34                       | 3                            | 32         | 65               |
| 4        | 21                       | 9                            | 9          | 81               |
| 6        | 9                        | 56                           | 22         | 22               |
| 8        | 22                       | 46                           | 41         | 13               |
| 23       | 8                        | 62                           | 12         | 25               |

* 0–5 grains.
† 6–19 grains.
§ 20 or more grains.
response, in both the primary and the secondary response. Statistical analyses were kindly performed according to the randomization test, a nonparametric statistical methods, by our colleague Dr. V. X. Gledhill. They showed that, for the primary response, the values for each of the day points 3, 4, and 5 did not differ significantly from each other; but each of these values was significantly higher than those for each of day 7 ($P$ values respectively being $< 0.05$, $< 0.01$, and $< 0.01$), days 9–10 ($P < 0.01$, $P < 0.01$, $P < 0.05$), or day 21 ($P < 0.05$, $P < 0.01$, $P < 0.01$). In the secondary response, values for days 6, 8, and 23 did not differ significantly, but the values for each of days 3 and 4 were significantly higher than for each of days 6, 8, and 23, $P$ values varying from $< 0.05$ to $< 0.001$. When the values for the “early” days 3–5 were pooled and compared with the pooled values of the “late” days 6–23, $P$ values considerably less than 0.0001 were obtained for either primary or secondary responses, and also for the combined primary and secondary response data.

The maturation of immunocytes is an asynchronous process, and it might be expected that, on any given day, some individual PFC would be less mature than others. Accordingly, the PFC were classified on the basis of their cyto logical appearance. Many cells were medium to large lymphocytes; others were inadequately flattened during the final step of micromanipulation. Thus only 102 cells could be confidently assigned to morphologic categories, the cell cycle characteristics of which were known from previous experience (4). Their labeling intensity is analyzed in Table II. The data confirm that “immature” PFC were more heavily labeled than “mature” PFC.

Grain counts of non-PFC showed that about one-half of the cells were unlabeled, presumably representing T cells which do not label under the conditions used (13). The other half showed a heterogeneous grain count distribution similar to that of the “early” PFC.

TABLE II

| Cell type                  | No. of single PFC examined | Per cent of cells |          |          |
|----------------------------|----------------------------|-------------------|----------|----------|
|                            |                            | Unlabeled | Lightly labeled | Heavily labeled |
| Blast                     | 26                         | 11       | 15         | 74       |
| Immature plasma cell      | 12                         | 0        | 16         | 84       |
| Mature plasma cell        | 50                         | 36       | 30         | 34       |
| Small lymphocyte          | 14                         | 65       | 21         | 14       |
This study shows that the amount of surface Ig on antibody-forming cells, as judged by binding of anti-Ig by cells at 0°C, is highly variable. While no difference between IgM-formers and IgG-formers sampled at equivalent times in the immune response was noted, there was systematic variation related to time after immunization and degree of cell maturity. Over-all, cells found early in primary and secondary responses, and cells judged to be immature, exhibited more surface Ig than cells persisting for long times after immunization or appearing to be mature. However, the finding was by no means universal, and exceptions in both directions were noted. The results were consistent with the view that receptors diminish in number, and presumably also in functional significance, with progressive maturation of immunocytes. Blast cells may remain responsive to further antigenic stimuli through their dense receptor coat, and mature cells, presumably fully programmed to synthesize antibody at maximal rate, may become antigen-independent entities through their loss of receptors. This view has been championed by both Pernis et al. (10) and McConnell (15). Some caution in interpretation may be advisable, however, because of the extreme cell-to-cell variation encountered, and the complex relationship existing between receptor numbers and amount of antireceptor bound per cell. For example, spacing of receptors, burying of the Fc portion, or steric hindrance may all influence the amount of anti-Ig bound to a given PFC. Alternatively, the findings may reflect a change not in receptor status but in the cell physiology of antibody secretion, with mature cells retaining less secretory product at the membrane than immature ones. Subject to these reservations, the findings may reveal yet one more level of regulation of the immune response.

**SUMMARY**

Spleen cells from CBA mice that had been primarily or secondarily immunized with sheep red blood cells were reacted at 0°C with a ^125^I-labeled polyvalent rabbit anti-mouse globulin reagent. After suitable washing, the cells were placed in a plaque-revealing monolayer and warmed to 37°C. Plaques appeared within 10–20 min. Single plaque-forming cells (PFC) were taken from the middle of plaques, were washed by micromanipulation, and were singly dried on glass slides. The amount of attached antireceptor was assessed by quantitative radioautography.

Great variation in “receptor density” was encountered among the 258 single cells studied. However, early, immature PFC in both primary and secondary responses had statistically significantly more receptors than late, mature PFC.
On any given day point, no difference was found between IgM- and IgG-
forming cells. The results were consistent with the view that cells still able to
be driven to further proliferation by antigen retain receptors, and conversely
that cells, as they mature, lose both receptors and ability to be influenced by
antigen.

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