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# ANTIGEN-INDUCED INCREASE IN THE NUMBER OF CELLS FORMING NONSPECIFIC IMMUNOGLOBULINS *in vitro*

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KEY WORDS: antibody-forming cells; cells forming nonspecific immunoglobulins; antigen; antibodies; nonspecific immunoglobulins.

The appearance of large quantities of nonspecific immunoglobulins (NIG) [2] during immunization has not yet been satisfactorily explained. This problem assumes particular importance in connection with the clonal selection theory, according to which the action of an antigen is selective in character.

This paper describes a study of the formation of cells producing antibodies (AFC) and NIG (NIGFC) during culture of lymphoid cells *in vitro*.

## EXPERIMENTAL METHOD

Spleen cells from C57BL/6 mice, intact or immunized 3-4 days before the experiment by intravenous injection of $5 \times 10^8$ sheep's red blood cells (SRBC), were used. Cell pools (from 4 to 5 spleens) were cultured for 1-4 days in a Mishell–Dutton system [8], in the modification described in [1, 4], in the presence of water-soluble SRBC antigen [11]. At the end of incubation the cells were washed 3 times with Eagle's medium containing 10% embryonic calf serum and used to determine the number of AFC [7] and of immunoglobulin-forming cells (IGFC) [9]. The number of NIGFC was calculated as the difference between the numbers of IGFC and AFC per $10^6$ living cells.

The experimental results were subjected to statistical analysis with calculation of the arithmetic mean ($M_A$) and the standard error ($\pm m$). Each point represents the mean of 15-27 parallel cultures.

## EXPERIMENTAL RESULTS

During culture of normal spleen cells for 1-4 days the mean survival rate of the cells until the end of incubation was 40%.

Addition of water-soluble SRBC antigen to the cell cultures induced a distinct immune response which reached a maximum on the 4th day. Besides an increase in the number of AFC in the cultures, the number of NIGFC also was increased. At the peak of the response (3rd day) the number of NIGFC was 25 times greater than initially (Table 1). The NIGFC/AFC ratio fell with an increase in the number of AFC in the samples.

It was shown previously [1, 5] that addition of antigen to a suspension of spleen cells from mice immunized 3-4 days previously with the same antigen causes an increase in the number of AFC, which is significantly greater than the number of AFC induced in cell suspensions from unimmunized animals. It was interesting to discover how preliminary immunization of animals *in vivo* would affect the increase in the number of NIGFC in cultures.

Experiments showed that under these circumstances many more NIGFC were formed than in cell suspensions from normal spleens (Table 1). The mean number of NIGFC reached 117,294/$10^6$ cells, but in one experiment the number of NIGFC was increased to 165,000 per $10^6$ cells, i.e., to 16.5% of the total number of cells in culture, equivalent to 33%

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TABLE 1. Changes in Ratio between Numbers of AFC and NIGFC during Culture of Mouse Spleen Cells with Antigen

| Source of cells | Duration of culture, days | Number of AFC per $10^6$ living cells | Number of NIGFC per $10^6$ living cells | NIGFC/AFC ratio | Increase in number of AFC | Increase in number of NIGFC |
|---|---|---|---|---|---|---|
| | | $M_A \pm m$ | | | | |
| Spleens of unimmunized animals | 0 (5) | | 2 027±729 | — | — | — |
| | 1 (22) | 1±0,3 | 3 313±334 | — | — | 1 286 |
| | 2 (23) | 25±4 | 11 112±876 | 445 | 24 | 9 085 |
| | 3 (27) | 297±35 | 51 218±3 253 | 172 | 296 | 49 191 |
| | 4 (15) | 1 730±108 | 43 706±3 556 | 25 | 1 729 | 41 679 |
| Spleens removed on 3rd day after injection of antigen into mice | 0 (5) | 99±11 | 3 538±188 | 36 | — | — |
| | 1 (11) | 381±23 | 3 558±215 | 9 | 282 | 20 |
| | 2 (11) | 1 662±172 | 26 110±1 158 | 16 | 1 563 | 22 572 |
| | 3 (11) | 5 221±440 | 117 294±14 836 | 23 | 5 122 | 113 756 |
| | 4 (9) | 5 258±738 | 36 283±5 768 | 10 | 5 159 | 32 745 |
| Spleens removed on 4th day after injection of antigen into mice | 0 (4) | 1 034±527 | 8 708±2 153 | 8 | — | — |
| | 1 (18) | 2 420±308 | 15 502±1 915 | 6 | 1 386 | 6 794 |
| | 2 (18) | 2 722±167 | 32 745±2 890 | 12 | 1 688 | 24 037 |
| | 3 (22) | 6 278±408 | 85 464±3 165 | 14 | 5 244 | 76 756 |
| | 4 (16) | 14 616±2 444 | 24 978±2 846 | 2 | 13 582 | 16 270 |

Legend. Number of observations in parentheses.

when counted as B cells. The increase in the number of NIGFC was observed as early as during the first day of incubation, it reached a maximum on the 3rd day, and thereafter fell sharply.

The decrease in the number of NIGFC was not due simply to death of the cells in suspension. The decrease in the number of cells between the 3rd and 4th days of culture did not exceed 16-31%, but the number of NIGFC fell by 69-71%. It must also be noted that the number of AFC during this period usually continued to rise. It can be concluded that the decrease in the number of NIGFC was due to selective inhibition of NIG formation or secretion by these cells, or to both processes together.

Density inhibition provides a very convenient model with which to study regulation of cell proliferation. In particular, inhibition of the rise in the number of AFC and of cell proliferation in high-density cultures has been demonstrated previously. This effect was much weaker in cultures of cells from immunized animals [6]. It was accordingly interesting to compare the effect of an increase in density on the number of NIGFC and on the NIGFC/AFC ratio in normal and "immune" suspensions at different times during cell culture. The formation of AFC and NIGFC was observed to take place both in optimal (4 million cells/ml) and in dense (20 million cells/ml) cultures. However, the number of AFC and NIGFC was considerably reduced in the high-density cultures. AFC formation was inhibited particularly sharply in high-density cultures of normal spleen cells. On the 4th day of culture *in vitro* the number of AFC in the dense cultures was $22 \pm 7/10^6$ living cells compared with $1856 \pm 313/10^6$ living cells in optimal cultures, i.e., 84 times less. The number of NIGFC fell by a much lesser degree: On the 4th day it was 20,153 ± 4147 in the dense cultures and $44{,}762 \pm 998/10^6$ living cells in the optimal cultures, i.e., only 2.2 times less.

In agreement with previous findings, density inhibition of AFC in cultures from the "immune" spleen was much weaker (by 13 times compared with 84) than in cultures of normal cells. Conversely, density inhibition of NIGFC either was not reduced or was actually stronger than in normal cultures.

The differences in the dynamics of AFC and NIGFC formation and the results of the density inhibition experiments indicate that AFC and NIGFC are different cell populations, whose formation is regulated by different methods.

What are these B cells which can be activated by antigen to synthesize NIG? They are evidently not AFC precursors (Table 1). It is also unlikely that they are cells producing antibodies with extremely low affinity [12]. A more likely explanation is that the NIGFC are either cells synthesizing antibodies against foreign antigens or a special cell population specifically triggered by a particular antigen and forming true NIG, the function of which is not yet clear.

The mechanism of stimulation of NIGFC by antigen may evidently be twofold: 1) They may be stimulated by the action of nonspecific stimulating factors produced by T-cells or macrophages under the influence of antigen (indirect action of antigen on B cells); 2) the B cells may be stimulated by the direct action of antigen on these cells, just as in the case of polyclonal activators of B cells [4, 10]. Some of the facts discovered in the present investigation are evidence in support of the second mechanism.

The problem of how many B cells undergo proliferation under the influence of this antigen is of fundamental interest. The answer can be obtained by analyzing curves showing the increase in number of AFC and NIGFC. If such curves are extrapolated to zero, it can easily be shown that during induction of the primary response *in vitro* the number of the initial cell population for NIGFC was $363/10^6$ living cells. Preliminary immunization of the animals with homologous antigen led to an increase in the initial population to 794-1895 cells per $10^6$ living cells.

It cannot be concluded from these results, it must be emphasized, that NIG producers, already present in the cell suspension, are stimulated to proliferate and differentiate (the number of NIGFC in normal and "immune" suspensions before the beginning of culture *in vitro* was 2027 and 3538-8707 per $10^6$ living cells, respectively) or that cells not previously synthesizing NIG are activated under the influence of the antigen. The solution to this problem can be obtained only from experiments with cell cultures from which the "background" NIGFC have been removed beforehand.

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# INCREASE IN NUMBER OF ANTIBODY- AND NONSPECIFIC IMMUNOGLOBULIN-FORMING CELLS IN MICE IMMUNIZED WITH T-DEPENDENT AND T-INDEPENDENT ANTIGENS


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**KEY WORDS:** T-dependent antigen; T-independent antigen; antibody-forming cells; cells forming nonspecific immunoglobulins; Vi-antigen; sheep's red blood cells.


It was shown previously that immunization of animals leads to an increase in the number not only of antibody-forming cells (AFC), but also of cells producing nonspecific immunoglobulins (NIGFC). The dynamics of the appearance of AFC and NIGFC has been found to differ [4, 10]. These results were obtained by the use of T-dependent antigens.

The object of this investigation was to study the dynamics of the increase in number of AFC and NIGFC after immunization of animals with T-independent antigen.

## EXPERIMENTAL METHOD

Female BALB/c mice weighing 14-16 g were used. Sheep's red blood cells (SRBC) were used as T-dependent antigen and *Salmonella typhi* Vi-antigen as T-independent antigen. The mice received one or two intravenous injections of $500 \times 10^6$ SRBC or 1 $\mu$g of Vi-antigen with an interval of 2-4 weeks. The spleens were removed on the 1st-7th days after the 1st or 2nd immunization and cell suspensions were prepared from them. Spleen cells from unimmunized animals were used in the control experiments. Usually cell pools from three to five spleens were used.

AFC were counted by direct and indirect local hemolysis methods [7], using native SRBC or SRBC sensitized with Vi-antigen [3] as the test antigens and rabbit antisera against mouse IgC (to detect indirect AFC against SRBC and Vi-antigen) and against the $\mu$-chains of mouse IgM (to detect AFC against Vi-antigen) as intensifying antisera.

Immunoglobulin-forming cells (IGFC) were determined with the aid of SRBC sensitized with rabbit antibodies against mouse IgG [9]. Antibodies against mouse IgG were isolated by affinity chromatography [2]. The number of NIGFC was


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