Interaction of Specific Antibody Precursor Cells in vitro with a Bacterial Somatic Antigen

There is much current interest concerning cellular events in antibody formation, especially the role and nature of antigen-reactive cells or antibody precursor cells prior to specific immunization. It is generally accepted that the first stages of the immune response may involve the recognition by specific leukocytes of an antigen, or at least an antigen that has first been processed by other cells[1,2]. A number of investigators have now demonstrated both in vitro interactions of a small proportion of lymphoid cells from non-immune animals with several different antigens[3–8]. The nature of such reactions and the role of lymphocytes that are involved in this phenomenon are not yet clear.

Pre-existing cells also are present in lymphoid organs which interact in vitro with particulate antigens such as xenogeneic erythrocytes or Gram-negative bacteria to form 'clusters' or rosettes[9–12]. Following specific immunization the number of such cluster- or rosette-forming cells increases significantly and reflects the intensity of the adaptive immune response[11–15]. However, the exact relationship between pre-existing cluster-forming cells to the immune response is largely unknown. Although it is unlikely that all or most of these cells are actually antigen-reactive or precursor cells[16–19], it seems reasonable that important information may be obtained by studying the effects of removing these cells from a spleen cell population. Destruction of leukocytes capable of reacting in vitro with a specific radio-isotope-labelled antigen has been found to decrease markedly the capability of a spleen cell suspension to transfer specific antibody formation to irradiated recipients[4–6]. In the present study cluster-forming cells to a bacterial somatic antigen pre-existing in the spleen of non-immunized mice[14,20,21] were physically removed by differential centrifugation and the remaining cells tested for their ability to adoptively transfer the immune response to other mice.

For these experiments 'normal' spleen cells were obtained from non-immunized NIH Albino A mice, washed carefully with buffered Hanks' solution in the cold, and adjusted to a standardized suspension. The cells were incubated in vitro at a ratio of approximately 1:100 with a suspension of heat-killed *E. coli* 0127B:8[9,14]. After 1 to 3 h incubation at 37 °C the spleen cell-bacteria suspensions were centrifuged in a 5 to 30% bovine serum albumin gradient in 3 mm diameter glass tubes. $^5/_{10}$ ml fractions were collected and total viable cell counts determined. The number of spleen cells forming clusters with adherent *E. coli* was determined for each fraction by microscopic examination[14,18,21]. In addition, the number of spleen cells interacting with bacteria so as to form bacterial adherent colonies (BAC) in subsequent agar gel cultures was determined for each fraction, exactly as described previously[20]. Portions of each fraction, as well as suitable controls, were then transferred i.v. to groups of 5 to 10 syngeneic recipient mice treated 24 h previously with whole body X-irradiation (850 R). Other mice were injected with unfractionated cell suspensions, or were given no cells as a control. As an additional control normal spleen cells were incubated as above with saline alone or with unrelated antigens such as heat-killed *Shigella* or sheep red blood cells. Each mouse was then challenged by i.p. injection of approximately 10^9 heat-killed *E. coli*. All mice were killed 8–9 days later and the number of BAC per spleen was determined. In addition, lytic antibody plaque-forming cells (PFC) to *E. coli* were also determined by the direct bacteriolytic plaque assay in agar gel exactly as described elsewhere[14,20].

As can be seen from the Table, incubation of normal spleen cell suspensions with *E. coli* in vitro, without subsequent centrifugation, had little effect on the appearance of BAC or PFC in recipient spleens. There was little

Effect of differential centrifugation of spleen cell suspensions on precursors of antibody forming cells to *E. coli* as assessed by transfer to antigen stimulated irradiated recipients

Spleen cell treatment [a] in vitro	Recipient antibody response [b] BAC ($\times 10^3$)	PFC ($\times 10^2$)	Serum titer [c] (mean) ($\log_2$)
None (saline controls)	13.7	36.5	6.3
E. coli – not centrifuged	15.1	32.6	6.7
Centrifuged – Fraction 1 (top)	1.8	34.5	2.3
2	1.2	36.9	3.5
3	3.1	33.1	3.9
4	9.3	37.5	7.3
Shigella – not centrifuged	14.1	31.5	6.9
Sheep RBC – not centrifuged	16.3	33.8	7.1
No cells transferred	<1.0	<1.0	<2

[a] Donor mouse spleen cells incubated for 60 min at 37 °C either alone or with indicated antigen; for fractionation cell-bacteria suspension centrifuged through 5–30% bovine serum albumin gradient. [b] Each recipient given $15–30 \times 10^6$ syngeneic spleen cells after indicated treatment and then challenged i.p. with *E. coli* vaccine; number of antibody forming cells per spleen (either bacterial adherent colonies or plaque forming cells) determined for 5–8 mice per group 8 days later. [c] Mean serum titer determined by bacteriolytic assay for each group by microtiter assay.

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or no difference between the responses of mice receiving donor spleen cells incubated with saline alone or with the *E. coli*. However, when *E. coli*-incubated spleen cell suspensions were fractionated by centrifugation, there were marked differences in the number of BAC appearing in recipient spleens (Table). Many fewer BAC were present when the upper fractions, which were devoid of most bacteria adherent cells, were used for cell transfer. In contrast, the lowest fraction, which contained many more bacterial cluster forming cells, generally gave 2–4 times more BAC. This number of BAC, however, was generally similar to that which occurred following transfer of similar numbers of splenocytes which had not been incubated in vitro with the *E. coli*. The number of bacteriolytic PFC to *E. coli* in recipient spleens did not vary significantly, regardless of the treatment of the donor spleen cell suspensions or the type of fraction transferred (Table).

Incubation of normal spleen cells with sheep erythrocytes or *Shigella* resulted also in clusters of spleen cells and specific antigen, but there was no detectable effect on the number of BAC appearing in recipient spleens (Table). In addition, only very low numbers of BAC and PFC appeared in the spleens of X-irradiated control mice that were not given donor spleen cells. The appearance of antibody-forming cells in the recipients could be attributed to the donor cell population. Viable cells were necessary, since heating of the donor splenocytes at 80 °C for 1 h, regardless of in vitro treatment with antigen, resulted in a marked diminution in the number of antibody-forming cells, either BAC or PFC, that appeared in the recipients. Thus, it seems unlikely that any antigen transferred with the spleen cell suspension influenced the recipient response. Only those recipients actively challenged with the *E. coli* had significant BAC or PFC responses.

It seems reasonable from the results of these experiments to conclude that some of the splenocytes from normal mice which react with *E. coli* in vitro to form clusters may be directly involved in the immune response, either as precursors of antibody-forming cells or as antigen-reactive cells. The separation of such reactive cells with adherent bacteria from spleen cell suspensions by centrifugation markedly affected the ability of the remaining cell population to adoptively transfer antibody-forming capacity to X-irradiated syngeneic mice, as assessed by the BAC assay. However, there was little effect on the PFC response of the same recipients, which suggests that precursor or antigen-reactive cells for BAC are different from those for PFC, which depend on synthesis of lytic antibody. Specificity of the reaction suggests there are separate and distinct pre-existing cell populations that can respond to specific antigens. Thus the spleen cells from non-immunized mice that reacted in vitro with *E. coli* appeared to be the same ones that were concerned with the adoptive transfer of BAC formation, but not with development of PFC. However, these cells may merely represent immunocytes that persist after earlier stimulation by the same or cross-reacting antigens in the environment. The removal of these cells from a spleen cell suspension could thus decrease the number of 'memory' cells necessary for a secondary, rather than a primary type response to this bacterial antigen.

Zusammenfassung. Es gelingt durch niedertourige Differentialzentrifugierung normaler Milzzellen der Maus, in vitro mit *E.-coli*-Suspensionen inkubiert, den Grobteil der Vorläufer Antikörper bildender Zellen abzutrennen, welche zusammen mit *E. coli* spezifische Agglutinine bilden konnten. Dies war durch Erfassung bakterieller «Kolonieverklumpung» in Empfänger-Mäusen zu bestimmen.

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Passive Hemagglutination of *Herpesvirus hominis*

The efficacy of the passive hemagglutination (PHA) test in detecting antibody to viral antigens is well established[1]. It was reported in the literature[2,3] that *Herpesvirus hominis* can be passively coated on erythrocytes which will then be agglutinated by specific immune sera. A more recent publication[4] confirmed the effectiveness of the PHA test for detecting antibodies to *Herpesvirus* and also indicated that this method is effective in distinguishing between the 2 antigenic types of virus. The purpose of the work herein reported was to separate the antigen responsible for the PHA activity and to further characterize this test.

Materials and methods. Herpesvirus, type 1 MacIntyre strain VR 3, was grown in both HeLa and HEp-2 cells. The cells were grown as monolayers in either prescription bottles or in roller bottles and were harvested when the cytopathic changes were well advanced (3+). Infected cells were washed at least 3 times, resuspended in balanced salt solution (BSS) and stored at −70 °C. Before they were used all preparations were centrifuged for 10 min at $1500 \times g$ to remove cellular debris. Certain preparations were stabilized with formaldehyde[5]. Viral preparations were either purified on 5% to 50% sucrose gradients and centrifuged at 30,000 to 50,000 $\times g$ for 1 h or spun to equilibrium in CsCl self-forming gradients at $100{,}000 \times g$ for 44 h[6].

Fractions were routinely collected by the bottom drip method and diluted with approximately 4 volumes of phosphate-buffered saline, pH 7.2 (PBS). In certain

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