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# CELLULAR AND HUMORAL EVENTS DURING HOMOGRAFT REACTION IN DIFFUSION CHAMBERS*

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Despite the numerous data available on homotransplantation there still exist several controversial problems, among others the fate of cells involved in homograft rejection, the role of a humoral, mobile factor, and the effect of sensitized cells upon target cells.

To study adequately the events that lead to the rejection of homografted cells one must have a system by which these processes can be studied systematically.

In approaching this problem Celada and Carter [1] devised an in-vivo culture method consisting of an irradiated $F_1$ hybrid as a test tube in which the rejecting capacity of varying numbers of a parent ($P_1$, C3H) strain spleen cells upon a second parent ($P_2$, C57BL) strain spleen cells, differing at the H-2 locus, could be assessed by using the depression of the secondary agglutinin response by $P_2$ spleen cells as a criterion for the rejection of $P_2$ cells and for the "killing" potency of $P_1$ cells.

However, this in-vivo test-tube method, although suitable for studying quantitatively the homograft-rejecting capacity of competent cells, does not permit precise cytological studies because cultured cells are not restricted to a limited area in the recipient animal. They proliferate among those of the recipient.

The requirement of a restricted culture area is fulfilled by the diffusion-chamber technique devised by Algire et al. and Prehn et al. [2, 3]. When cultured in this porous-membrane chamber, the cells are kept separate from those of the recipient. Furthermore, several reports on the cytokinetics of the immune response and homograft studies indicate that this technique is suitable for studying systematically the cellular changes that occur during the immune response [4-12].

In this study the Celada-Carter method and a modified version of the diffusion-chamber technique [4] were combined to investigate, during homograft reaction, the fate of killer and target mouse spleen cells placed in single-or double-compartment diffusion chambers with a porosity of 0.1 $\mu$m, intraperitoneally implanted into sublethally irradiated, immunologically inert mice. Preliminary results have been reported earlier [13].

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## MATERIALS AND METHODS

### Animals

Twelve-week-old mice of the following inbred strains and $F_1$ hybrids from Cumberland View Farm were used for most of these experiments: C3H/Anf Cum (H-$2^{kk}$), C57BL/Cum (H-$2^{bb}$), and (C57BL/Cum ♀ × C3H/Anf Cum ♂) $F_1$, to be referred to as $P_1$, $P_2$ and $F_1$ respectively. For some experiments inbred strains and $F_1$ hybrid from the Radiobiology Department of the Atomic Energy Commission were used: C3H/Ep, C57BL/Ep and (C57BL/Ep ♀ × C3H/Ep ♂) $F_1$, to be referred to as $P_1$, $P_2$ and $F_1$ respectively.

### Irradiation factors

A GE-300 and a Phillips X-ray machine were used as the sources. For the former the conditions were 300 kVp, 20 mA; 190 R/min at 70 cm; HVL, 0.478 mm Cu; inherent filtration 4.75 mm Be; added filtration, 2mm Al. For the second: 200 kVp; 15 mA; 65 R/min at 60 cm; added filtration 1 mm Al plus 0.5 mm Cu. Recipient mice were placed in a circular perforated Lucite container attached to a revolving turntable, and a single total-body exposure of 500 R was given about 6 h before diffusion chambers were implanted intraperitoneally.

### Homograft-reaction assay in diffusion chambers

The method devised by Celada and Carter [1] was used, except that the "killing" effect of spleen cells from $P_1$ mice on "target" spleen cells from $P_2$ or $F_1$ mice were observed in diffusion chambers implanted intraperitoneally into X-rayed $P_1$ or $F_1$ mice.

Generally, for primary homograft reaction studies, target cells were obtained from $F_1$ mice, and for secondary homograft reaction studies from $P_2$ mice. In the latter case $P_1$ mice were pre-immunized intraperitoneally with $2 \times 10^7$ $P_2$ spleen cells about a week before the removal of their spleen. In all cases $P_2$ or $F_1$ mice were immunized intraperitoneally with 1 ml of 1% rat red blood cells (RBC) suspension about two weeks before the removal of their spleen. As described previously [4], a desired concentration of cell suspension was inoculated into sterile single-or double-compartment diffusion chambers (SCDCh. and DCDCh.). These chambers were made of Lucite rings. The double-compartment diffusion chambers were built from two lucite rings with three walls of Millipore filters (a half chamber attached to a complete one). SCDCh. were inoculated with $12\text{-}24 \times 10^6$ target and/or killer spleen cells and $3 \times 10^6$ rat RBC. When DCDCh. were used, $24 \times 10^6$ killer $P_1$ spleen cells were inoculated into one of the compartments, and 24 million target $P_2$ or $F_1$ spleen cells and three million rat RBC antigen into the other compartment. The following control-chamber studies were run: (a) Blank chambers, (b) killer $P_1$ spleen cells only, (c) target $P_2$ or $F_1$ spleen cells plus rat RBC, (d) target $P_2$ or $F_1$ spleen cells only, (e) rat

RBC only, (f) killer $P_1$ spleen cells plus rat RBC, and (g) target $P_2$ spleen cells plus $P_2$ spleen cells from donors not immunized against rat RBC, plus rat RBC.

With use of single-compartment diffusion chambers, spleen cells were labelled with tritiated thymidine (H3T) (1 mCi/ml, specific activity 0.36 Ci/mmol, Schwarz Laboratories) in situ. $P_1$ mice were injected intraperitoneally twice with 15 $\mu$Ci of H3T within a 30-min interval. One hour after the second injection of H3T their spleens were removed and processed. $P_2$ mice were also given two intraperitoneal injections of H3T, the first with 10 $\mu$Ci and 20 h later the second with 20 $\mu$Ci. Also, one hour later, their spleens were removed and processed. With use of the double-compartment chambers, spleen cells were labelled by injecting 5 $\mu$Ci of H3T intraperitoneally into the recipient mice.

Diffusion chambers were removed from the X-rayed recipient mice at varying intervals after implantation, or after an intraperitoneal injection of H3T into the recipient mice, the earliest taking place within an hour after treatment. The chamber fluid was assayed for anti-rat RBC titre and smears were stained with Giemsa, or subjected to autoradiography by using NTB3 Kodak nuclear track emulsion (exposure time 8 d). After development these smears were stained with Giemsa through the emulsion according to Gude's method [14].

The proliferative capacity of lymphoid cells was determined by sampling the percentage of H3T-labelled cells at varying time intervals. The number of cells counted per time interval was about one thousand per chamber. For these experiments mouse spleen cells were grouped as follows: Reticulum-blast cells, (R-B), immature lymphocytes (IL), immature plasmacytes (IP), mature lymphocytes (ML), mature plasmacytes (MP), histiocytes (H), phagocytes (Ph), immature and mature granulocytes (IG-MG) and erythroblasts (Eb). By multiplying the percentages of each cell type obtained with differential counts, with the total number of nucleated cells per chamber at a given interval, data on the total number of each type of cells per chamber at that time was obtained. With the same procedure the absolute number of H3T-labelled cells of a given type of cell per chamber per time interval was obtained.

## GENERAL SCHEME OF THE EXPERIMENTS

Two different criteria were used to evaluate the fate of killer and target cells cultured in diffusion chambers. One was the degree of depression of secondary agglutinin response by target cells when cultured with killer cells, and the second was the proliferative rate of both type of cells determined by means of the tritiated thymidine labelling technique. We know from previous work [15] that when pre-immunized spleen cells, injected intravenously into a heavily irradiated (unresponsive) isologous recipient, are secondarily stimulated with the test antigen, there is, during the logarithmic phase of antibody production, a linear relationship between $\log_2$ antibody titre and cell number. The slope of the regression line is 1.0. Consequently it is possible to estimate the

cell number in terms of antibody response relative to that of the non-treated cells. Also we demonstrated that mouse spleen cells inoculated either intravenously into heavily irradiated isologous recipients, or into diffusion chambers, proliferate with a mean doubling time of 24 h, as indicated by the increase in the percentage of H3T-labelled lymphoid cells and the decrease in the mean grain count per cell during culture [4-16]. Furthermore, we found that when these cells are primarily or secondarily stimulated with an agglutinogen (sheep red blood cells) there exists, during the latent and the logarithmic phases, a two-fold decrease in the mean generation time compared with that of the non-stimulated cel

With these approaches we devised several experiments to study adequately the fate of killer and of target cells during the homograft reaction and also the existence of a mobile factor responsible for the rejection of the homologous antigens.

In the first set of experiments we used single-compartment diffusion chambers. To investigate the fats of both killer and target cells two experiments were done. In one, killer cells from H3T-labelled donors were cultured with 12 million target spleen cells from unlabelled donors. In the second 12 million killer cells from unlabelled donors were cultured with the same amount of target spleen cells from H3T-labelled donors. With this experiment the "killing" effect of killer cells upon target cells was investigated. Killer-cell activity was also studied by determining the relative antibody response by pre-immunized target cells when cultured with pre-sensitized or non-sensitized killer cells as compared with that of the target cells cultured without killer cells. The relative antibody forming activity was determined by the formula:

$$\text{R.Ab.F.Act.} = \frac{1}{2s(T_c - T_{exp})} \times 100$$

where: s = slope = 1.0, $T_c = \log_2$ titre of control group, and $T_{exp} = \log_2$ titre of experimental group. For this purpose 12 million killer spleen cells from pre-immunized or non-immunized $P_1$ donors were cultured with 12 million $P_2$ or $F_1$ target cells pre-immunized against rat RBC plus three million rat RBC and the agglutinin titre recorded at 4 and 5 d of culture.

For the second set of experiments double-compartment diffusion chambers were used. As in single-compartment-chamber studies, both the proliferative rate of killer and target cells and the effect of killer cells upon the agglutinin response of target cells were investigated. For the first purpose 24 million killer cells from pre-immunized or non-immunized donors were cultured in one compartment, and 24 million target spleen cells from pre-immunized or non-immunized donors in the opposite compartment. At varying time intervals after implantation of the chambers, recipient irradiated mice were injected with H3T.

For the second purpose 24 million killer spleen cells from pre-immunized or non-immunized donors were cultured in the same manner as in the previous experiment, with $P_2$ or $F_1$ target spleen cells from donors pre-immunized against rat RBC, plus three million rat RBC.

The chambers were intraperitoneally implanted into 500 R treated $P_1$ mice, one chamber per mouse. Five days after culture the chambers were removed and the anti-rat titre determined in the chamber fluid samples by the two-fold dilution method.

## RESULTS AND DISCUSSION

### Single-compartment diffusion-chamber studies

Figure 1 shows the proliferative rate of killer and target spleen cells labelled with H3T in situ. As can be seen, while pre-sensitized killer cells proliferate with a mean doubling time of about 12 h, the number of labelled target cells decreases sharply during the first 24 h of culture. On the other hand, target cells cultured without killer cells, the control group, proliferate normally with a mean doubling time of about 24 h. Killer cells from non-sensitized donors cultured without target cells proliferate also with a mean doubling time of about 24 h. This observation was not plotted.

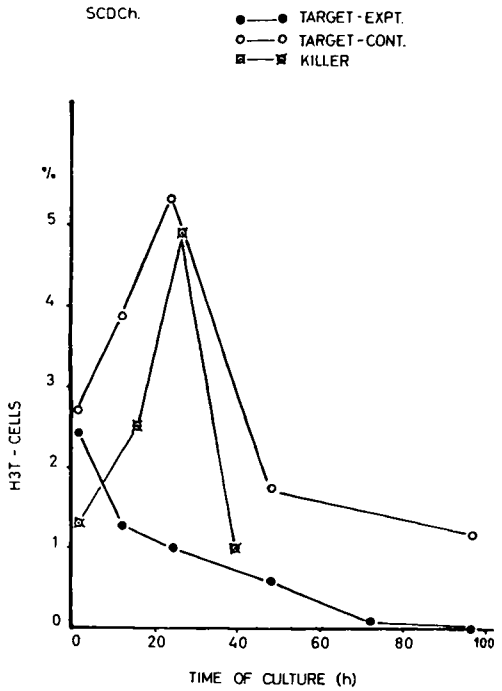


FIG. 1. Proliferative rate of H3T-labelled killer $P_1$ spleen cells pre-sensitized against $P_2$ antigens, and H3T-labelled target $P_2$ spleen cells cultured in SCDCh. Control-group chambers contained only $P_2$ cells.

In previous work done on the proliferative rate of mouse spleen cells [4, 5, 16] we found that non-stimulated spleen cells proliferate with a doubling time of 24 h, while stimulated cells proliferate during the latent

and logarithmic phases of antibody response with a doubling time of abou 12 h. In the present experiments the accelerated proliferation rate of killer cells could be indicative of antigenic stimulation while the decreas in the number of H3T-labelled target cells in the experimental group is indicative of cell destruction and/or arrested proliferation.

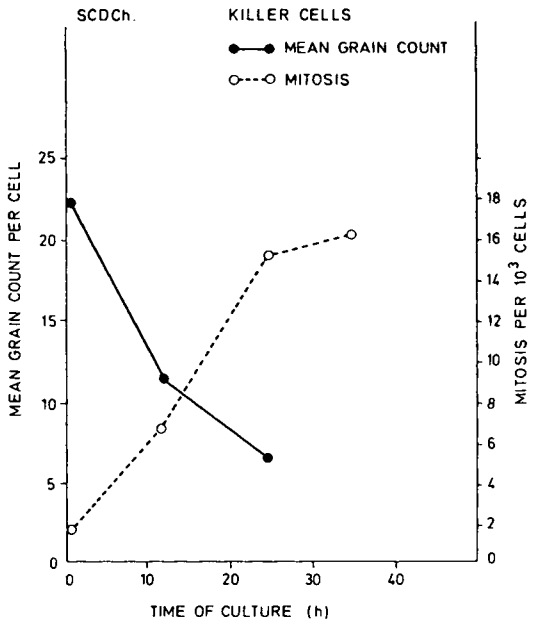


FIG. 2. Proliferation of in-situ H3T-labelled killer $P_1$ spleen cells during homograft reaction in SCDCh. Mean grain count and mitotic index.

The active proliferation rate of killer cells was also estimated by determining the mean grain count per cell. The results are shown in Fig. 2. It can be observed that the mean grain count decreased to a half in about 12 h. Accordingly, the number of mitotic cells increased with time after culture.

Table I shows the results on the effect of killer spleen cells upon the immune response capacity of target cells. In this experiment 12 million killer spleen cells sensitized with $P_2$ antigens two days earlier were cultured, together with 12 million target $P_2$ spleen cells primed against rat RBC plus three million rat RBC. The results show a great decrease in the immune response by target cells on the fifth day of culture. In terms of relative activity 94% of the target cells were destroyed. Comparative results when pre-sensitized or non-sensitized $P_1$ spleen cells were cultured with $P_2$ or $F_1$ spleen cells respectively are shown in Fig. 3. It is possible to see a depressive effect of non-sensitized $P_1$ spleen cells on the immune response capacity of $F_1$ hybrid. In this case, in terms of relative antibody activity, 86% of the target cells were destroyed.

TABLE I. SECONDARY AGGLUTININ RESPONSE BY TARGET $P_2$ SPLEEN CELLS CULTURED WITH OR WITHOUT KILLER $P_1$ SPLEEN CELLS IN SCDCh.

| Time after incubation (d) | Anti-rat agglutinin titre ($log_2$)[a] | |
|---|---|---|
| | Target cells alone | Target cells plus killer cells |
| 4 | 5.00 | 3.60 |
| 5 | 8.20 | 4.20 |

[a] Chamber fluid samples

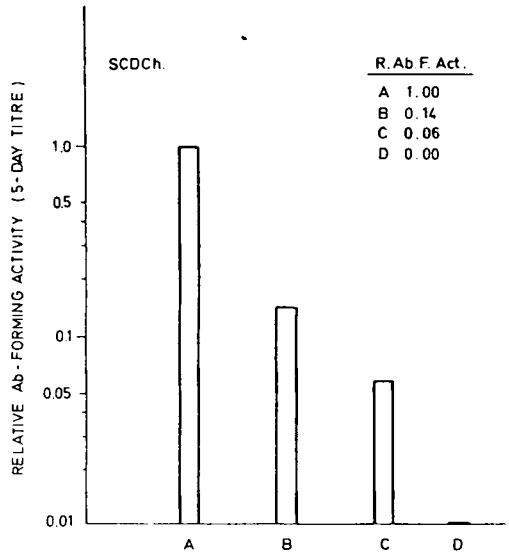


FIG. 3. Effect of pre-sensitized and non-sensitized killer $P_1$ spleen cells upon the immune capacity of $P_2$ or $F_1$ spleen cells pre-immunized against rat RBC assayed on the fifth day of culture in SCDCh.
A = primed $P_2$ or $F_1$ spleen cells plus rat RBC. R. Ab. F. Act. 100%.
B = $F_1$ spleen cells cultured with non-presensitized $P_1$ spleen cells. R. Ab. F. Act. 14 %.
C = primed $P_2$ spleen cells cultured with pre-sensitized $P_1$ cells. R. Ab. F. Act. 6%.
D = killer $P_1$ cells cultured with rat RBC. R. Ab. F. Act. 0%.

## Double-compartment diffusion-chamber studies.

By using the single-compartment diffusion chamber it was possible to carry out cytological studies during homograft reaction of both killer

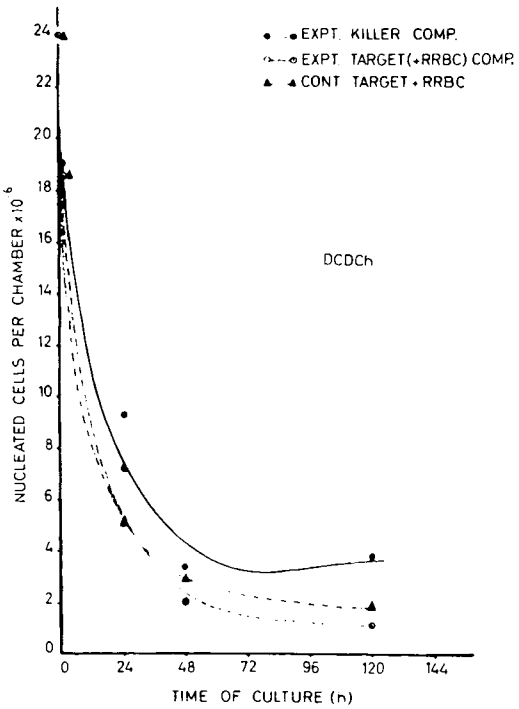


FIG. 4. Number of free recoverable spleen cells per chamber with time after culture in DCDCh. during homograft reaction. Four samples per point.

and target H3T-labelled spleen cells in an adequate manner because, in this system, cultured cells are kept separated from those of the recipient animal. However, because in this model the killer and target cells are morphologically similar (spleen cells), precise cytomorphological studies of each cell population during the homograft reaction are not possible because both types of cells are cultured together. In seeking a system whereby killer and target cells can be kept separated from each other during the homograft reaction, we devised a double-compartment diffusion chamber (see material and methods). In this chamber both cell populations remain separated from each other by means of a porous membrane impermeable to nucleated cells. With this model we intended to study the morphological changes occurring in the killer and in the target compartments providing that killer cells can be sensitized by target antigens through the filter membrane, and that killer cells produce a mobile factor which can diffuse through the porous membrane and react with target cells.

As in previous experiments we first determined the number of recoverable cells cultured in each compartment during the time after culture. Figure 4 shows the results of this experiment. The experimental group chambers were filled with 24 million spleen cells from C3H donors sensitized to $P_2$ (C57BL) spleen cells in one side and, on the opposite,

24 million $P_2$ spleen cells immunized against rat RBC plus three million rat RBC. Control-group chambers contained 24 million target $P_2$ cells plus three million rat RBC, on one side and few drops of Tyrode solution in the other compartment. Samples were taken at 1, 24, 48 and 120 h after chamber implantation. It was observed that, immediately after implantation, there was a sharp decrease in the total number of free recoverable cells per chamber of each compartment in the experimental as well as in the control group, representing, in 48 h of culture, about 12% of the initial inoculum. However, by the fifth day after culture, different figures were obtained for each compartment. Thus, average numbers of 1.1, 1.9, and 3.8 million were obtained for target experimental, target control, and killer compartment respectively.

In most experiments with double-compartment diffusion chambers we considered data obtained on the second and fifth days after culture for proliferative and cytomorphological studies, and data on the fifth day only for studies on the degree of depression by killer cells of the secondary agglutinin response by target cells.

In one experiment, performed to investigate the proliferative rate of killer and target cells, cultured cells were labelled one hour after chamber implantation by an intraperitoneal injection of 5 $\mu$Ci of H3T into the irradiated recipient mice. The percentages of labelled cells in each compartment was determined in samples taken at 1, 12, 24, 36, and 48 h after labelling. In this case, as was found with SCDCh., killer cells proliferate with a mean doubling time of 12 h. The same doubling time was observed for target cells of the control group undergoing a secondary anti-rat response. This agrees with the results we obtained previously [4-5]. But, on the contrary, the proliferative rate of target cells of the experimental group showed a marked decrease and the number of H3T-labelled target cells decreased considerably during the first 24 h after culture and labelling.

Figure 5 shows the ratio between the total number of H3T-labelled target cells of the experimental group per chamber to the total number of labelled cells of the target compartment in the control group, on samples taken 1, 12, 24, 36 and 48 h after pulse labelling. A decrease in the number of labelled target cells per chamber in the experimental group compared with those of the control group was observed. These results indicate the existence of a mobile factor produced by killer cells, capable of diffusing through the filter membrane and exerting a destructive effect upon target spleen cells cultured in the opposite compartment.

Figure 6 shows the results obtained with the experiments performed to investigate, by means of the degree of depression of the immune capacity of target cells, the existence of a diffusible homograft-rejection factor and the sensitization of killer cells without having close contact with target cells. Two reaction models were considered. The first pre-sensitized $P_1$ cells against $P_2$ cells and the second had no pre-sensitized $P_1$ cells against $F_1$ spleen cells, i.e. a secondary and a primary homograft reaction, respectively. In terms of relative activity 89% of the

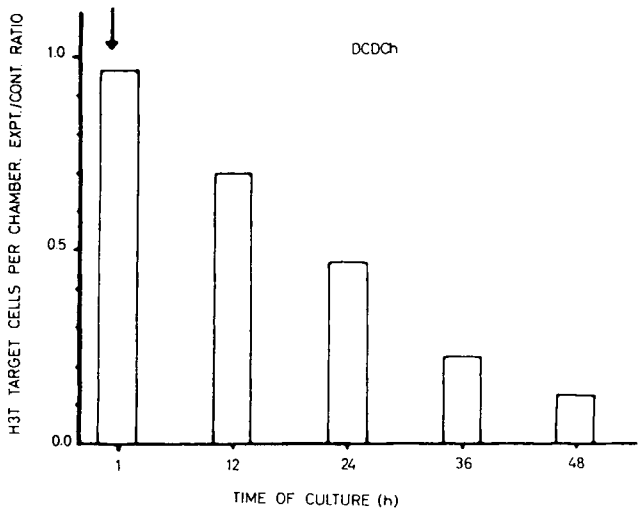


FIG. 5. Fate of H3T-labelled target $P_2$ spleen cells cultured in DCDCh. as expressed by the ratio H3T-target cells per chamber of the experimental group/H3T-labelled target cells per chamber of the control group (without killer cells). Arrow indicates labelling of cultured spleen cells with H3T.

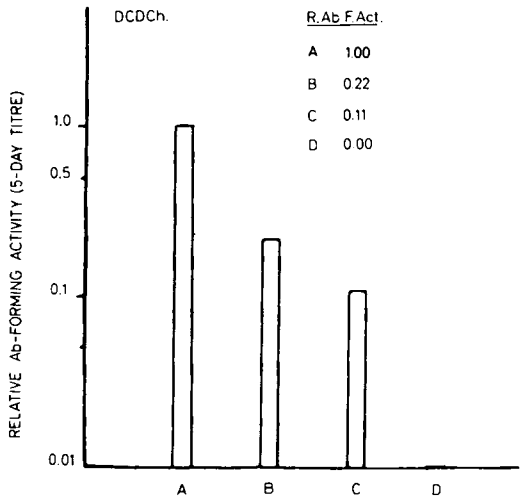


FIG. 6. Effect of pre-sensitized and non-sensitized killer $P_1$ spleen cells upon the immune capacity of $P_2$ or $F_1$ spleen primed with rat RBC assayed on the fifth day of culture.
A = primed $P_2$ or $F_1$ spleen cells undergoing secondary agglutinin response and cultured without killer cells (control group). R. Ab. F. Act. 100%.
B = primed $F_1$ spleen cells cultured with non-sensitized $P_1$ spleen cells. R. Ab. F. Act. 22%.
C = primed $P_2$ cells cultured with primed $P_1$ cells. R. Ab. F. Act. 11%.
D = $3 \times 10^6$ or $6 \times 10^6$ RBC in DCDCh. or blank chambers. R. Ab. F. Act. 0%.

target cells were destroyed during secondary reaction and 78% during the primary reaction on the fifth day of culture.

With this system then, primary sensitization of competent spleen cells was obtained without mediating in the cell-to-cell contact with homologous cells.

These results agree with those of Dvorak and Waksman [17] and of Najarian and Feldman [18].

With the knowledge that the killer cells, when cultured together or separated by means of a porous membrane from target cells, provoke a depression of the proliferative and the immune capacities of target cells, we wished to ascertain to what extent this depression was due to cell destruction or to the inhibition of protein synthesis. We present data concerning the first question.

In Fig. 7 are represented the variations in number of cells per chamber per cell type in the target compartment during primary homograft reaction as compared with the control group on the second and fifth days of culture.

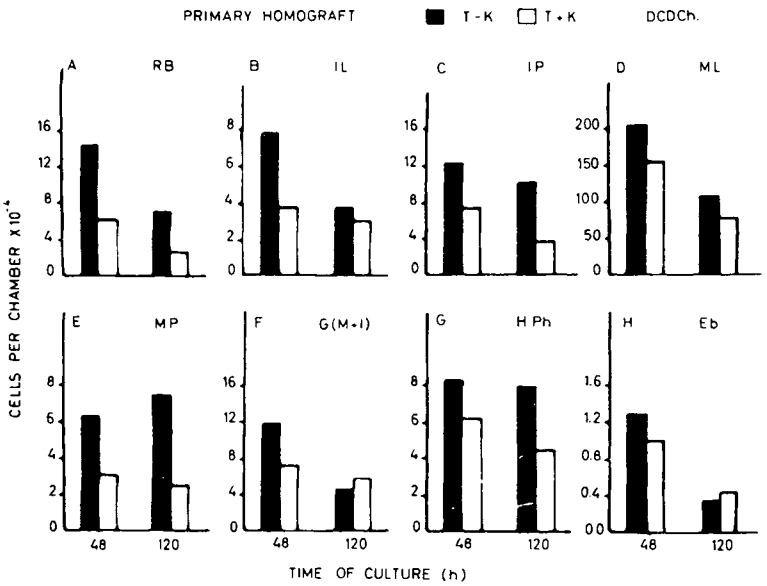


FIG. 7. Primary homograft reaction in DCDCh. Variation in the number of target cells per chamber recovered at 48 and 120 h after culture. R-B = reticulum blast cells; IL = immature lymphocytes; IP = immature plasmacytes; ML = mature lymphocytes; MP = mature plasmacytes; G = mature and immature granulocytes; H-Ph = histiocytes and phagocytes; Eb = erythroblasts. ▭ Experimental group. ▬ Control group.

A significant decrease is observed among reticulum-blast-cells, immature plasmacytes and mature plasmacytes groups as compared with the controls. This difference is more pronounced on the fifth day, specially among mature plasmacytes where about a three-fold decrease is observed.

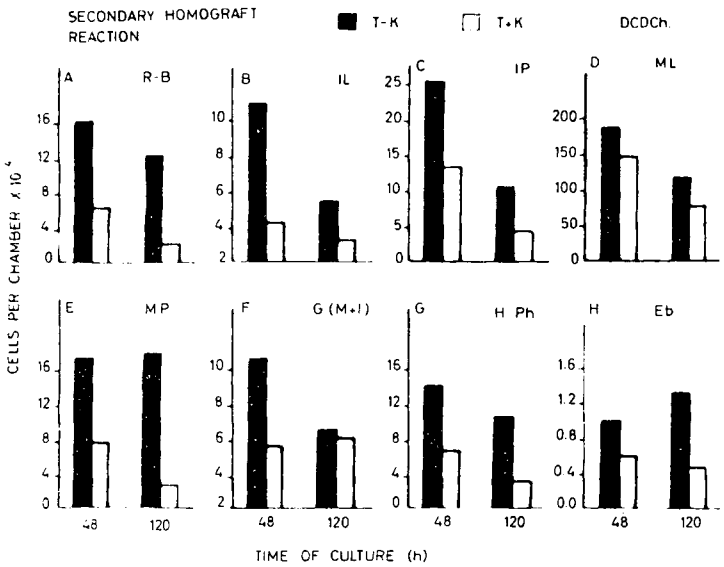


FIG. 8. Secondary homograft reaction in DCDCh. Variation in number of target cells per chamber recovered at 48 and 120 h of culture. (R-B, IL, IP, ML, MP, G, H-Ph, Eb, are listed in the caption of Fig. 7). ▭ Experimental group. ▬ Control group.

On the contrary, compared with the control group, granulocytes increase within this time. This was also true for erythroblasts, although in this group the sample size was too small to validate this result.

Figure 8 shows the results of a similar experiment during secondary homograft reaction. Here, as expected, the decrease of target cells is more accentuated than in the primary. The greatest decrease, about six-fold, is found among blast-cells and mature plasmacytes. Granulocytes do not vary compared with the control group. A significant decrease, about three-fold, is also seen among hystiocytes and phagocytic cells.

Tables II and III show the absolute numbers per cell type per chamber in the target compartment of experimental and control groups on the fifth day of culture during primary and secondary homograft reaction, respectively.

A better estimation of the decrease in the number of target cells is seen in Fig. 9 where the ratio of cell number per chamber per cell type between the experimental and the control group is represented during primary and secondary reactions on the fifth day of culture. In over-all decrease that of the reticulum-blast cells and the mature and immature plasmacytes predominate.

In general it can be seen that the effect of the diffusible factor in this system is mainly upon blast- and lymphoidal-type cells. Granulocytes remain unaltered both in the primary and in the secondary reaction.

Figure 10 shows the ratio of H3T-pulse-labelled target cells per cell type per chamber between experimental and control groups on the fifth

TABLE II. NUCLEATED CELL NUMBER ($\times 10^{-3}$) IN TARGET COMPARTMENT DURING PRIMARY HOMOGRAFT REACTION ON FIFTH DAY OF CULTURE IN DCDCh.

| Cell type | R-B | IL | ML | IP | MP | IG | MG | H-Ph | Eb |
|---|---|---|---|---|---|---|---|---|---|
| Control[a] | 73.2 | 39.0 | 1290.0 | 114.4 | 74.2 | 11.7 | 36.0 | 81.9 | 3.7 |
| Experimental[b] | 29.3 | 30.5 | 810.0 | 39.6 | 25.5 | 14.5 | 45.1 | 45.6 | 4.4 |

[a] Average of 15 samples

[b] Average of 16 samples

TABLE III. NUCLEATED CELL NUMBER ($\times 10^{-3}$) IN TARGET COMPARTMENT DURING SECONDARY HOMOGRAFT REACTION ON FIFTH DAY OF CULTURE IN DCDCh.

| Cell type | R-B | IL | ML | IP | MP | IG | MG | H-Ph | Eb |
|---|---|---|---|---|---|---|---|---|---|
| Control[a] | 118.8 | 54.4 | 1172.7 | 108.0 | 173.0 | 13.0 | 51.6 | 107.0 | 13.0 |
| Experimental[b] | 31.0 | 32.5 | 835.0 | 43.0 | 26.0 | 21.0 | 39.5 | 30.0 | 44.0 |

[a] Average of 13 samples

[b] Average of 14 samples

day of culture. In agreement with the previous experiment a high decrease is observed among blast cells and lympho-plasmacytic cells during the primary and the secondary reaction.

If we consider the absolute number of cells per chamber (Fig. 7) a two-to three-fold decrease in reticulum-blast cells and lymphoplasmacytic cells was observed in the experimental group as compared with that of the control group. However, when only H3T-labelled cells of the same type are considered a four-fold decrease was observed. This difference suggests that the diffusible homo-rejecting factor in some degree affects the DNA synthesis process of target cells.

If the reduction of agglutinin titre were only due to cell loss then, according to the values obtained on the absolute number of nucleated cells per chamber on the fifth day (See Tables II and III) in R-B, IM and

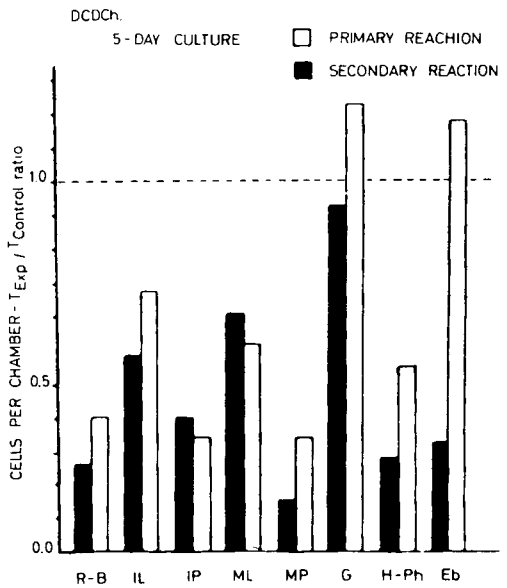


FIG. 9. Destructive effect of killer $P_1$ spleen cells on the different morphological types of $P_2$ or $F_1$ target spleen cells on the fifth day of culture in DCDCh. as expressed by the ratio, experimental/contro group. (R-B, IL, IP, ML, MP, G, H-Ph, and Eb are listed in the caption of Fig. 7.)

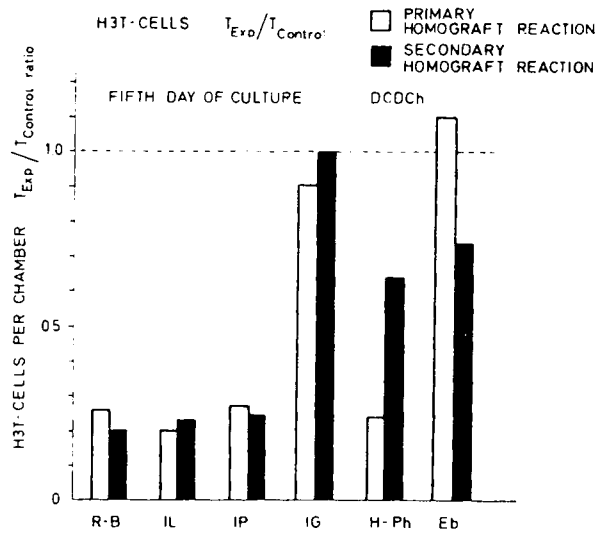


FIG. 10. Effect of killer $P_1$ spleen cells upon the number of H3T-labelled $P_2$ or $F_1$ target spleen cells per chamber on the fifth day of culture in DCDCh. as expressed by the ratio experimental over the control group. R-B, IL, IP, ML, MP, G, H-Ph, and Eb are the same as the Figs 7, 8 and 9. Samples were taken one hour after H3T injection.

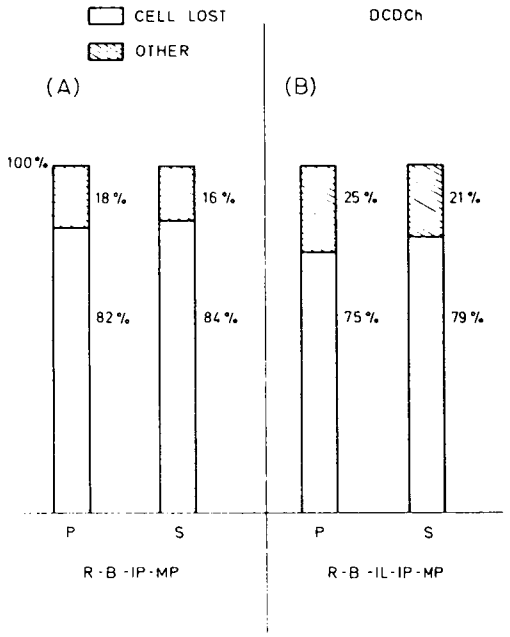


FIG. 11. Participation of cell destruction on the total depression of the secondary agglutinin response by target $P_2$ or $F_1$ spleen cells when cultured in DCDCh. with pre-sensitized or non-sensitized $P_1$ spleen cells. P = primary homograft reaction; S = secondary homograft reaction. R-B, IL, IP, and MP are the same as in Fig. 7.

MP groups, and relating cell number to antibody-forming activity, the depression of the immune response on the primary and the secondary reaction should be equal to 64% and 75%, respectively. However, the actual values of the depression of the immune response were higher (78% and 89%, respectively). Thus, we can assume that, out of the total depression of the agglutinin-forming activity by target cells, 80% is due to cell loss and 20% to other causes such as the inhibition of protein synthesis.

This assumption is expressed in Fig. 11, in which the corresponding values are given in Fig. 11 (A), taking into consideration the values on reticulum-blast cells and mature and immature plasmacytes, and in Fig. 11 (B) if the immature lymphocytes are included. In both cases the values are very close.

Figure 12 shows the variation in number per chamber per cell type in the killer compartment expressed by the ratio of the experimental to the control group. A five-fold increase occurs on the fifth day of culture among the reticulum-blast cells and histiocytes as compared with the control group. A two-and-a-half times increase is observed in immature plasmacytes with a slightly higher value for immature lymphocytes. Among mature plasmacytes only a one-and-a-half times increase compared with the control was observed.

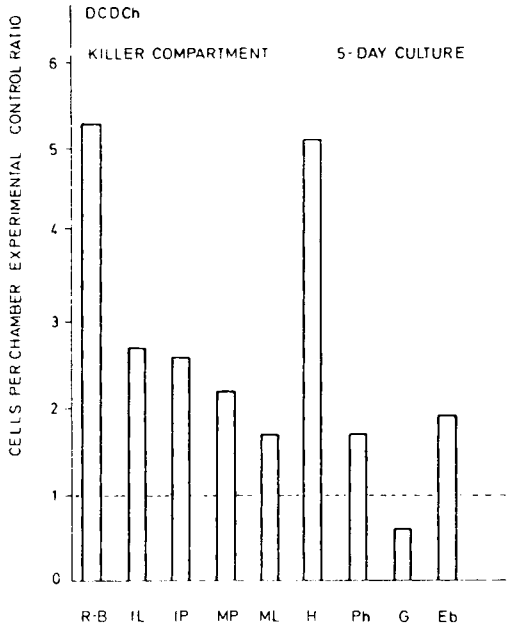


FIG. 12. Relative change in the proportion of cell types between experimental and control groups in killer comparment. R-B, IL, IP, ML, MP, G, H-Ph, and Eb are the same as in Fig. 7.

This differs from what we have observed in previous experiments on cytokinesis of mouse spleen cells during secondary agglutinin response (anti-sheep RBC) [6] where a four-fold increase was observed among plasmacytes on the fifth day of culture in diffusion chambers.

In agreement with the results of André et al. [19] the present observation suggests that, in the rejection of allogeneic nucleated cells, the blast cells, histiocytes and lymphocytes play the major role, rather than the plasmacytes. This is supported by experiments in which the fate of the killer and target cells was followed for more than 10 days after culture. There was no significant increase of plasmacytes by that time.

## SUMMARY

Using single-and double-compartment diffusion chambers, studies were performed on homograft reaction by mouse spleen cells differing at the H-2 locus. Target cells are destroyed and probably functionally inhibited (anti-rat response) when cultured in contact with killer cells or separated from them by a porous membrane. However, killer cells proliferate at a higher rate than non-stimulated cells as was seen in the immune response. Evidence is reported on primary sensitization of competent spleen cells against homologous cells without mediating cell-to-cell contact.

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## SUMMARY OF DISCUSSION

The use of the term homograft for the double-compartment diffusion chamber was discussed at length since the mechanism seems to be that of a classical humoral immune response and probably different from the mechanism included in, for instance, the destruction of a skin graft.