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Peritoneal exudate T lymphocytes with specificity to sheep red blood cells

II. INFLAMMATORY HELPER T CELLS AND EFFECTOR T CELLS IN MICE WITH DELAYED-TYPE HYPERSENSITIVITY AND IN SUPPRESSED MICE

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Summary. Peritoneal exudate cells were induced in mice 4 days after immunization with SRBC. A low dose of SRBC (10^6 i.v.) caused T lymphocytes to appear in inflammatory exudates. These cells, not only transferred DTH reactions, but also functioned as helper T cells in antibody production after transfer to syngeneic nu/nu recipient mice. After a high dose of SRBC (10^9 i.v.), very few helper T cells and no DTH transferring T cells were found in inflammatory exudates, although they were present in the spleen. It is postulated that T cells mediating DTH reactions and helper T cells behave similarly as far as the dose dependency of appearance in inflammatory exudates is concerned. A high dose of sensitizing antigen causes retention of helper and effector T cells in the spleen, in this way favouring antibody formation; low doses of antigen allow them to leave the spleen, thus favouring mediation of DTH reactions in the periphery.

INTRODUCTION

Humoral and cellular immunity to sheep red blood cells (SRBC) are inversely related, depending on the dose of antigen used for sensitization (Lagrange, Mackaness & Miller, 1974). After the injection of low doses of SRBC, delayed-type hypersensitivity (DTH) ensues, whilst after increasing the dosage of antigen, DTH disappears and antibody production rises. Yet, T cells are involved in both types of immune response, acting either as helper cells in antibody formation (Claman, Chaperon & Tripplett, 1966) or as effector T cells in the mediation of DTH reactions (Miller, Mackaness & Lagrange, 1973). It has been suggested (Kettman, 1972) that the T cells which mediate the two phenomena both belong to the same subpopulation of thymus-derived cells.

We have recently demonstrated (Hahn, Kaufmann, Miller & Mackaness, 1979) that effector T cells mediating DTH to SRBC enter inflammatory exudates. This finding has made it possible to study whether helper T cells also leave the site of their production in the spleen and enter peripheral inflammatory exudates.

The following report summarizes experiments which demonstrate that helper T cells appear in peripheral inflammatory exudates in parallel with effector T cells, under conditions which lead to DTH, and that suppression after high doses of antigen causes committed T cells, both helper and effector, to disappear from the periphery.

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MATERIALS AND METHODS

Mice

B6D2F1 mice, C3H mice, and nu/nu mice of C3H background were bought from Bomholtgård, Ry, Denmark, and were used at 6 weeks of age and older. Only mice of the same sex were used in a given experiment.

SRBC-specific T lymphocytes

Sensitization of donor mice and production of SRBC-specific peritoneal exudate cells (PEC) have been described before (Hahn *et al.*, 1979). In brief, donor mice were sensitized with 10^6 or 10^9 SRBC i.v., respectively. Casein exudates were induced on day 4 of the immune response and PEC collected 2 days later. For the collection of spleen cells, spleens were removed aseptically and squeezed through stainless steel sieves. The cells were filtered through gauze and purified on nylon wool columns (Julius, Simpson & Herzenberg, 1973). Treatment of cells with anti-Thy 1.2 serum and complement was done as described by Hahn *et al.* (1979) by using a 1:3000 dilution of anti-Thy 1.2 serum (Olac Ltd, Bicester, England) and a 1:10 dilution of agarose-absorbed guinea-pig serum.

Transfer of DTH

Transfer of cells mediating DTH reactions and test for DTH have been described previously (Hahn *et al.*, 1979). In brief, 2×10^7 PEC or 5×10^7 nylon non-adherent spleen cells from SRBC-sensitive donor mice were transferred to non-sensitized syngeneic recipient mice. Challenge injections of 1 or $2{\cdot}5 \times 10^8$ SRBC in a volume of 0·05 were made s.c. into one hind footpad. The degree of swelling was measured 24 h later and expressed in units of 0·1 mm.

Assay for helper cell activity

Syngeneic nu/nu mice were injected with 1 or 2×10^7 PEC and simultaneously sensitized with 10^8 SRBC i.v. Indirect plaque-forming cells (PFC) were determined 8 days later, using the Jerne–Nordin technique as modified by Finger & Emmerling (1968), by the addition of rabbit anti-mouse gamma globulin (Nordic Immunological Laboratories).

Statistical evaluation

The Wilcoxon rank test was used for statistical evaluation of the data. Medians and ranges are shown in the tables.

RESULTS

Relationship between cellular and humoral immune response in SRBC-sensitive mice

When a low dose of SRBC (10^6 i.v.) was employed for sensitization, good DTH reactions resulted, and DTH could be transferred to normal recipient mice using PEC (Table 1). On the other hand, when mice were sensitized with a high dose (10^9 i.v.), there was no measurable DTH in cell donors and DTH could not be transferred with PEC, indicating that some kind of suppression had arisen which not only prevented DTH, but also the appearance of DTH-mediating cells in inflammatory exudates (Table 1).

The humoral immune response, as measured by the indirect PFC response in the spleen 8 days after sensitization, behaved inversely to the cellular response: lower numbers of indirect PFC occurred after 10^6 SRBC i.v. than after 10^9 SRBC i.v. (Table 1).

Despite a low humoral response in mice sensitized by low doses of SRBC, peritoneal exudates from mice with DTH contained helper T cells for antibody production, as is shown by the following experiments.

Helper T cells and T cells mediating DTH in peripheral exudates of mice with DTH to SRBC

Mice were sensitized with 10^6 or 10^9 SRBC i.v. On day 4 of the immune response, peritoneal exudates were induced as usual. PEC were collected 48 h later and 2×10^7 viable PEC from either group of donors transferred to syngeneic nu/nu recipient mice. Afterwards, a sensitizing dose of 10^8 SRBC was injected i.v. and the number of PFC determined 8 days later. The biological activity of PEC was confirmed, not only by their helper cell activity, but also by measuring DTH reactions in a separate group of syngeneic cell recipients. PEC from mice sensitized with 10^6 SRBC exerted good helper cell activity, whereas PEC from mice sensitized with 10^9 SRBC exerted little helper cell activity (Table 2).

The data in Table 3 show that inflammatory helper cells are Thy 1.2 positive and hence are T cells.

Transfer of DTH with spleen cells from mice sensitized with a high dose of SRBC

Our data so far have demonstrated that helper T cells, able to assist in antibody production, are present in the periphery of mice with DTH to SRBC, despite the fact that such mice do not produce much antibody. On the

other hand, after high doses of antigen (i.e. when DTH is suppressed), indirect PFC in the spleen are high and therefore helper T cells must be present in the spleen. Thus, it appears likely that in mice sensitized with high doses of SRBC, committed T cells are retained in the spleen and thereby prevented from entering the circulation. This applies, not only to helper T cells, but also to DTH-mediating effector T cells, since it could be shown in the following experiment that T cells mediating DTH reactions occur in spleens of mice sensitized with high doses of antigen.

Mice were sensitized by injecting 10^9 SRBC i.v. and 4 days later, 5×10^7 nylon non-adherent viable spleen cells were transferred to non-immunized recipient mice. The latter were challenged with $2{\cdot}5 \times 10^8$ SRBC in one hind footpad 1 day after cell transfer and DTH reactions measured 24 h later. The results, shown in Table 4, in agreement with those of Askenase, Hayden

Table 1. PFC and DTH in donor mice and DTH transferred by PEC after sensitization with either a low or high dose of antigen ($B6D2F_1$ mice)*

Group	Sensitization	Indirect PFC per donor spleen ($\times 10^{-3}$)†	Donor DTH (0·1 mm)†	DTH Transferred by 2×10^7 PEC (0·1 mm)†
A	10^6 SRBCi.v.	3·2 (1·6–3·4)	10·0 (9·5–11·0)	5·0 (4·5–7·0)
B	10^9 SRBC i.v.	25 (16–37)	2·0 (2·0–2·5)	2·0 (1·0–2·0)
C	None	2·1 (1·0–2·8)	1·0 (1·0–2·0)	1·0 (1·0)

* Challenge was made immediately after transfer using 1×10^8 SRBC.
† Medians and ranges (in parentheses), $n=5$, significant differences ($P<0{\cdot}001$). PFC: A *v.* B; B *v.* C. Donor DTH: A *v.* B; A *v.* C. DTH transferred: A *v.* B; A *v.* C.

Table 2. Helper cell activity of PEC from mice sensitized with a high dose or with a low dose of SRBC (C3H mice)

Group	Sensitization of donors	nu/nu mice received	Indirect PFC per spleen ($\times 10^{-3}$)*
A	—	10^8 SRBC	4·0 (1·0–5·0) ($n=4$)
B	10^6 SRBC i.v.	10^8 SRBC + PEC†	170·0 (140–200) ($n=6$)
C	10^6 SRBC i.v.	PEC†	1·3 (1·0–2·0) ($n=6$)
D	10^9 SRBC i.v.	10^8 SRBC + PEC†	25·0 (15–52) ($n=6$)
E	10^9 SRBC i.v.	PEC†	1·1 (0·4–1·8) ($n=6$)

* Medians and ranges (in parentheses), Significant differences ($P<0{\cdot}01$): A *v.* B; A *v.* D; B *v.* C; B *v.* D; B *v.* E; C *v.* D; D *v.* E.
† 20×10^6 PEC per recipient.

Table 3. Anti-Thy 1.2 sensitivity of peritoneal exudate helper cells from donor mice which had been sensitized with 10^6 SRBC (C3H mice)

Group	Treatment of PEC	nu/nu mice received	Indirect PFC per spleen ($\times 10^{-3}$)*
A	None	10^8 SRBC + PEC†	161·0 (134–172)
B	anti-Thy 1.2 + Complement	10^8 SRBC + PEC†	0·9 (0·6–1·4)
C	Complement	10^8 SRBC + PEC†	144·0 (121–156)
D	—	10^8 SRBC	1·8 (1·2–2·2)

* Medians and ranges (in parentheses), $n=5$. Statistical differences ($P<0{\cdot}01$): A *v.* B; A *v.* D; B *v.* D; B *v.* C.
† 10×10^6 PEC per recipient.

Table 4. Transfer of DTH with 1×10^8 spleen cells from donor mice which had been sensitized with low or high doses of antigen (B6D2 mice)*

Group	Donor sensitization	Donor DTH (0·1 mm)†	Recipient DTH (0·1 mm)†
A	10^6 SRBC i.v.	14·0 (12·5–15·0)	10·0 (8·5–11·0)
B	10^9 SRBC i.v.	3·0 (2·5–4·0)	10·0 (8·0–12·0)
C	None	1·0 (1·0–2·5)	1·5 (1·0–2·5)

* Challenge was made 1 day after cell transfer using $2{\cdot}5 \times 10^8$ SRBC.
† Medians and ranges (in parentheses), $n = 5$. Statistical differences ($P < 0{\cdot}01$): Donor DTH: A *v.* B; A *v.* C. Recipient DTH: A *v.* C; B *v.* C.

& Gershon (1977), clearly demonstrate that effector T cells, able to transfer DTH reactions, are present in spleens of mice sensitized with high doses of SRBC.

DISCUSSION

The reported data demonstrate that helper T cells occur in the periphery of mice optimally sensitized for DTH. They enter inflammatory exudates as is the case with effector T cells, too, which mediate DTH reactions (Hahn *et al.*, 1979). Both helper T cells and T cells mediating DTH make their appearance in peripheral exudates only under conditions which favour cellular immunity, i.e. after low doses of sensitizing antigen (Lagrange *et al.*, 1974). It has been shown (Playfair, 1972) that sensitization with low doses of antigen preferentially stimulates T cells, whereas high doses are necessary to induce antibody-forming B cells. After high doses of antigen, however, DTH reactions can no longer be elicited (Lagrange *et al.*, 1974) and, concomitantly, neither T cells able to transfer DTH reactions nor helper T cells are any longer demonstrable within inflammatory exudates (Table 2). Nevertheless, helper T cells are still present in the sensitized mouse after a high dose of sensitizing antigen as must be inferred from their involvement in antibody production within the spleen. Effector T cells with the ability to mediate DTH reactions could also be shown to be present in spleens after high doses of sensitizing antigen (Table 4). Thus, it appears that helper T cells and effector T cells mediating DTH reactions are retained in the spleen when a high dose of antigen has been employed for sensitization, and for this reason are no longer available in the periphery.

These are common features which suggest that helper T cells and T cells mediating DTH reactions in the periphery are identical, as does the fact that both DTH reactions and helper T-cell activity in antibody production are mediated by $Lyl^+, 2^-$ T cells (Cantor & Boyse, 1975; Huber, Devinsky, Gershon & Cantor, 1976; Hahn *et al.*, 1979). Other authors (Kettman, 1972) have reached a similar conclusion, although the question has not been definitely settled (Snell, 1978).

High doses of antigen not only induce B cells, but also activate suppressor mechanisms acting on T cells (Ramshaw, Bretscher & Parish, 1976; Mackaness, Lagrange, Miller & Ishibashi, 1974). It appears probable that the latter are responsible for the inverse relationship which exists between cellular and humoral immunity, as proposed in the following hypothesis: helper T cells and T cells mediating DTH reactions are identical. High doses of antigen activate suppressor mechanisms which keep committed $Ly\,1^+, 2^-$ T cells within the spleen, where they act as helper T cells in antibody production and are unavailable in the periphery to mediate DTH reactions. After low doses of antigen, suppressor mechanisms are not activated, and therefore committed T cells can leave the spleen, enter the circulation, and are available in the periphery to mediate DTH reactions and act as helper T cells peripherally. Thus, suppression in this model would work, at least in part, by changing the anatomical distribution of committed T cells. Further experiments to prove this hypothesis are under way.

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