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Peritoneal exudate T lymphocytes with specificity to sheep red blood cells

I. PRODUCTION AND CHARACTERIZATION AS TO FUNCTION AND PHENOTYPE

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Summary. T lymphocytes which mediate DTH reactions to sheep red blood cells (SRBC) in mice enter casein-induced peritoneal exudates from which they can be recovered and assayed in a passive transfer system. Peritoneal exudates need not contain specific antigen for inducement of T-cell immigration. The amount (or biological activity) of DTH-transferring peritoneal exudate lymphocytes is enhanced by the previous use of immune modulating agents, such as cyclophosphamide (Cy) (200 mg/kg 2 days prior to sensitization), or BCG (10^7 live organisms i.v. 14 days prior to sensitization). SRBC-specific peritoneal exudate lymphocytes phenotypically are Thy 1^+ and Ly 1^+, 2^-. *In vivo,* peritoneal exudate T cells from Cy-modulated donors persist in circulation for a short period only and are subject to the suppressive mechanisms acting in anergic mice. Cells from BCG-plus-Cy-modulated donors, on the other hand, persist in circulation for a longer period and appear to be less susceptible to immune suppression.

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INTRODUCTION

Antigen-specific T cells, although their identity was unknown at the time, were first shown by Landsteiner & Chase (1942) to occur in peritoneal exudates of guinea-pigs displaying delayed-type hypersensitivity (DTH). Later studies, notably by Asherson and co-workers (Asherson, Allwood & Mayhew, 1973) have established the T-cell nature of specific exudate cells, and Asherson & Allwood (1972), in order to account for the exudate seeking property of such cells, have coined the term 'inflammatory lymphocytes'.

Recently, the immune response to sheep red blood cells (SRBC) in mice has become a widely used model for the study of the immune regulatory mechanisms, since it has been shown that either a humoral or cellular response can be induced according to the mode of sensitization (Lagrange, Mackaness & Miller, 1974).

Using this model, we have produced SRBC-specific inflammatory T lymphocytes with the aim in mind of characterizing the T cells which enter exudates with respect to their biological activities and surface markers. This report deals with the means of produc-

tion and some of the properties of specific inflammatory lymphocytes which mediate DTH to SRBC in mice.

MATERIALS AND METHODS

Animals

Unless otherwise stated, (C57Bl/6 × DBA/2) F_1 (B6D2) mice of either sex were used at 5–6 weeks of age.

Sensitization and immune modulation

SRBC (Biologische Arbeitsgemeinschaft, Lich, FRG) were stored in Alsever's solution at 4°, washed before being used and diluted to the appropriate concentrations in sterile 0·9% NaCl solution. SRBC were injected intravenously (i.v.) into the tail vein in a volume of 0·2 ml, or subcutaneously (s.c.) into one hind foot pad in a volume of 0·05 ml.

Cy (Endoxan ®, Asta, Brackwede, FRG) was dissolved in sterile water before use, and 2 days before sensitization, 0·2 ml was injected i.v. to give a dosage of 200 mg/kg.

BCG (Pasteur strain as propagated at the Trudeau Institute, Saranac Lake, N.Y., U.S.A.) had been fresh frozen and was kept at −70°. Mice were infected by the i.v. route with approximately 1×10^7 viable BCG per mouse 14 days before sensitization with SRBC. Counts were verified by plating the inoculum on Middlebrook 7H10 medium and counting bacterial colonies 3 weeks later.

Test for DTH

DTH was measured as described by Miller, Mackaness & Lagrange (1973). In short, challenge injections of 10^8 SRBC were made s.c. into one hind footpad and the degree of swelling measured 24 h later, using a dial gauge caliper ('Schnelltaster', H.C. Kröplin, Schlüchtern, FRG). The difference between sham-injected and antigen-injected footpads served as a measure of DTH (units of 0·1 mm). Routinely, challenge controls were done by injecting non-immune mice, in which the degree of swelling never exceeded 2 units.

Collection and transfer of peritoneal exudate cells (PEC)

On the day of the optimal immune response, mice were injected i.p. with 2 ml of a 5% casein solution. PEC were harvested 24 or 48 h later by rinsing the peritoneal cavities once with 3 ml of Hanks' tissue culture medium 199 (Flow Laboratories, Bonn, FRG), containing 1% foetal calf serum, 5 units/ml heparin, 5 units/ml penicillin, and 5μg/ml of streptomycin. Cells were washed, counted, and their viability determined by trypan blue exclusion. Desired numbers of washed cells were suspended in 0·2 ml of medium and injected i.v. into recipient mice. Numbers of transferred cells were either expressed as definite numbers or as 'peritoneal equivalents', one peritoneal equivalent representing the total number of PEC contained in 3 ml of washout. One equivalent thus represents the cellular immune power recoverable from a peritoneal exudate of one sensitized mouse at a given time after sensitization.

Nylon wool enrichment of exudate T lymphocytes

T lymphocytes were enriched from PEC by the method of Julius, Simpson & Herzenberg (1973). Briefly, nylon wool columns (about 5 g nylon wool/column) were preincubated with medium for 60 min (37°, 5% CO_2) and cell suspensions (1×10^7 PEC/ml) washed into the columns using warm (37°) medium. Subsequently, columns were incubated for 45 min (37° 5% CO_2) and carefully rinsed with warm medium. Effluents were collected, washed, and cell concentrations adjusted as necessary.

Treatment with anti-Thy 1·2 serum

Anti-Thy 1·2 serum and agarose-absorbed guinea-pig serum were a gift of Dr R. J. North, Trudeau Institute, Saranac Lake, N.Y., U.S.A. Serum had been prepared as described by North & Spitalny (1974). Before transfer, 1×10^8/ml washed PEC were incubated in anti-Thy 1·2 serum (diluted 1:5) for 30 min at 37°. Subsequently, cells were washed twice, resuspended in the same volume of guinea-pig complement (diluted 1 : 5), and incubated for 30 min at 37°. Control cells were treated with normal AKR serum and complement.

Treatment with anti-Ly sera

Anti-Ly 1·1 and anti-Ly 2·2 sera were the kind gift of Drs E. A. Boyse, U. Hämmerling, and F. W. Shen, Memorial Sloan-Kettering Cancer Center, N.Y., U.S.A. For details of the preparation and use of antisera see Shen, Boyse & Cantor (1975). T cells were enriched from PEC by incubation in nylon wool columns. Afterwards, 0·5 ml aliquots of cell suspension were incubated in 1 : 20 diluted antiserum at a cell concentration of 1×10^8/ml at room temperature for 30 min. After washing, cells were resuspended in the

same volume of 1 : 10 diluted rabbit complement of low cytotoxicity and incubated at 37° for 45 min. Cells were washed twice, cell concentrations adjusted as desired, and cells transferred into normal recipient mice.

Anergic mice

Mice were rendered anergic by injecting 10^9 SRBC i.v. 4 days before serving as recipients for adoptive transfer of reactive PEC.

RESULTS

Transfer of DTH to SRBC by PEC from non-modulated mice

Figure 1 shows the dose response relationship obtained after injecting various doses of SRBC i.v. and challenging for DTH on day 4 of the immune response. Maximal DTH responses were obtained with 10^6 SRBC.

For production of PEC, a pool of prospective donor mice was sensitized by the i.v. injection of 10^6 SRBC. Starting on day 2 of the immune response until day 6, DTH was measured daily in five representative mice. Peritoneal exudates were induced in parallel groups of mice and PEC, collected 24 h after induction, and transferred at a 1 : 1 donor to recipient ratio. Challenge was done immediately after cell transfer and DTH reactions measured 24 h later. Figure 2 represents the kinetics of DTH in the donors and the adoptively sensitized recipients. PEC induced on day 4 of the immune response transferred maximum DTH. Transferable DTH rapidly declined thereafter.

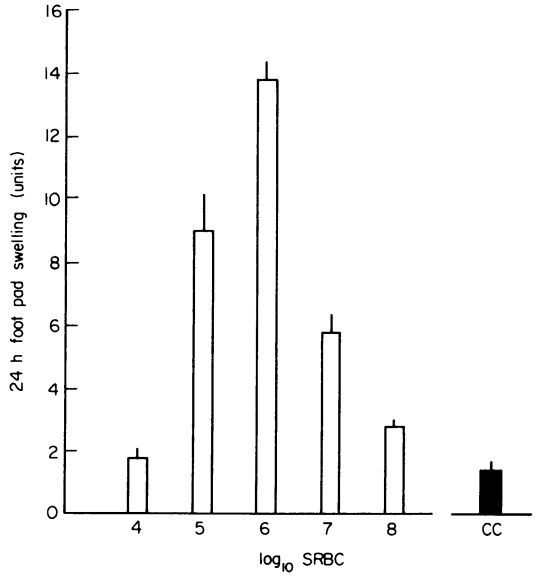

Figure 1. DTH in mice sensitized i.v. with various doses of SRBC and challenged on day 4 of the immune response. Challenge control (CC) denotes level of DTH in non-sensitized mice. Groups of five mice each ± SE.

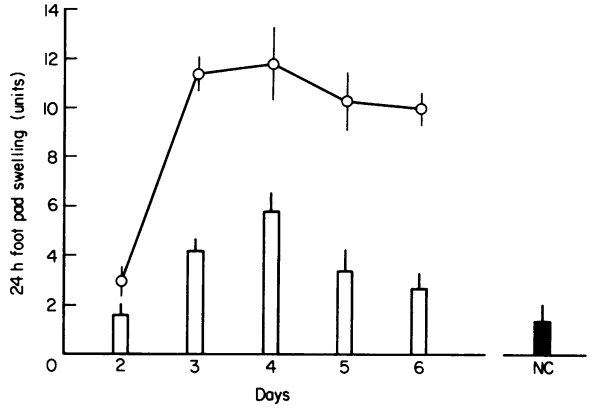

Figure 2. Transfer of DTH to SRBC by PEC from mice sensitized by 10^6 SRBC given i.v. Peritoneal exudates were induced on the days indicated. Cells were harvested 24 h later, and 1 peritoneal equivalent transferred per non-sensitized mouse which were challenged immediately thereafter. NC, DTH elicited in recipients of PEC from non-sensitized mice. Curve depicts donor DTH. Groups of five mice each ± SE.

Augmentation of transferable DTH by immune modulation with Cy and BCG

Since modulation of the immune response by Cy as well as by BCG plus Cy results in augmented DTH (Lagrange & Mackaness, 1975), it was next tried to increase the levels of DTH transferred by PEC by immune modulation of cell donors. Following a protocol similar to the one adhered to in the previous experiment, mice were sensitized by the i.v. injection of 10^8 SRBC, given 2 days after Cy-modulation. Exudates were induced on days 3, 5 and 9, harvested and transferred 48 h later at a 2 : 1 donor to recipient ratio.

Figure 3 shows that mice modulated by Cy consistently yielded exudates which transferred higher levels of DTH than exudates from mice sensitized with 10^6 SRBC.

Even greater levels of DTH could be transferred by PEC obtained from mice which had been given 10^7 live BCG, 14 days, and Cy, 2 days before i.v. sensitization with 10^8 SRBC (Fig. 4). For instance, one single peri-

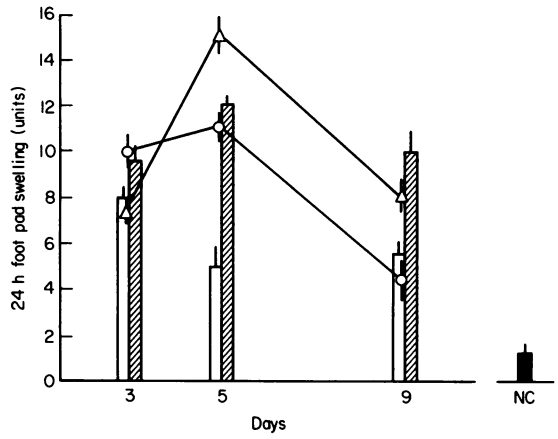

Figure 3. Transfer of DTH to SRBC by PEC from mice sensitized either by 10^6 SRBC i.v. (open bars) or by 10^8 SRBC i.v. after Cy-modulation (hatched bars). Exudates were induced on the days indicated and transfers made at a 2:1 donor/recipient ratio 48 h after exudate induction. o, DTH in donors sensitized with 10^6 SRBC; Δ, DTH in donor mice sensitized under Cy-modulation. Five mice per group ± SE.

toneal equivalent of PEC induced on day 7 or day 9 of the immune response transferred no less than 19 units of DTH, thus reflecting the augmenting effect of BCG plus Cy on the T-cell response to SRBC.

Similar results were obtained when mice were immunized (either under Cy or under BCG plus Cy) subcutaneously in the footpad (results not shown).

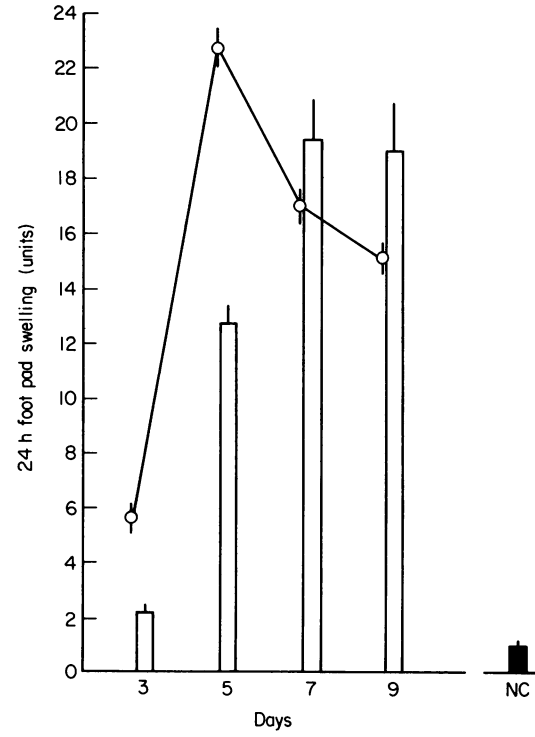

Figure 4. Transfer of DTH to SRBC by PEC from mice sensitized by 10^8 SRBC i.v., given under BCG-plus-Cy-modulation. Exudates were induced on the days indicated. Cells were collected 24 h later and transferred at a 1:1 donor/-recipient ratio. o, donor DTH. For further details see legend to Fig. 2. Groups of five animals ± SE.

SRBC-specific PEL are Thy 1^+ lymphocytes

PEL were collected from donors which, in the particular experiment depicted in Fig. 5, had been immunized locally 5 days before exudate induction by intra-footpad injection of 10^8 SRBC under Cy-modulation. After washing, PEC were subjected to anti-Thy 1·2 serum treatment as described under materials and methods before being injected into recipients. 3×10^7 anti-Thy 1·2 serum-plus-complement-treated cells (group C) expressed no more DTH than background *v.* 9·1 units transferred by 3×10^7 untreated cells (groups A and B) which indicates that the cells in PEC active in DTH transfer are Thy 1^+ lymphocytes (PEL).

SRBC-specific PEL are Ly $1^+, 2^-$

PEL were produced in CBA and C57Bl/6 donors which, in appropriate preliminary experiments, had been shown to respond in a similar fashion to SRBC as B6D2 mice. PEL were enriched by incubation in nylon wool columns. PEL from C57Bl/6 mice were incubated with anti-Ly 2·2 serum plus complement or without antiserum. PEL from CBA mice were incubated with anti Ly 1·1 serum plus complement or without antiserum. After incubation, PEL were washed and 3×10^6 cells transferred to syngeneic recipients. Treatment with anti-Ly 1·1 serum reduced the DTH transferring capacity of PEL from CBA mice. On the other hand, treatment with anti-Ly 2·2 serum did not reduce the DTH transferring capacity of PEL from C57Bl/6 mice (Fig. 6). Thus, PEL mediating DTH to SRBC are Ly $1^+, 2^-$.

Differential longevity of PEL from BCG- and non-BCG-modulated donors

DTH to SRBC fades rapidly unless BCG is used as an

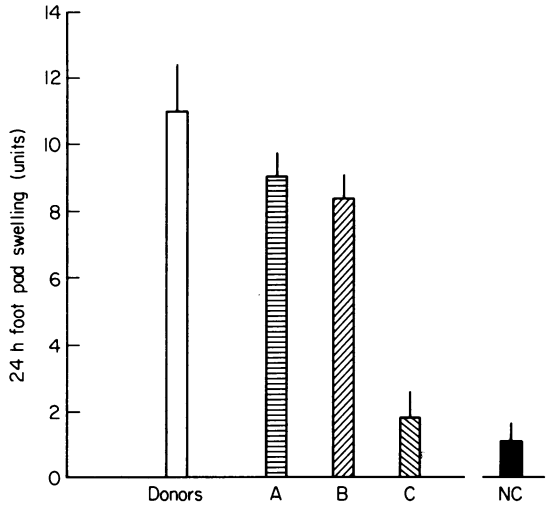

Figure 5. Sensitivity of DTH transferring PEL to anti-Thy 1·2 serum. PEC were collected from donors sensitized by intrafootpad injection of 10^8 SRBC under Cy-modulation, 5 days before exudate induction. Cells were either untreated (group A), treated with normal AKR serum plus complement (group B), or treated with anti-Thy 1·2 serum plus complement (group C). 3×10^7 cells were transferred per recipient. Five recipients per group ± SE. NC, recipients of PEC from non-sensitized donors.

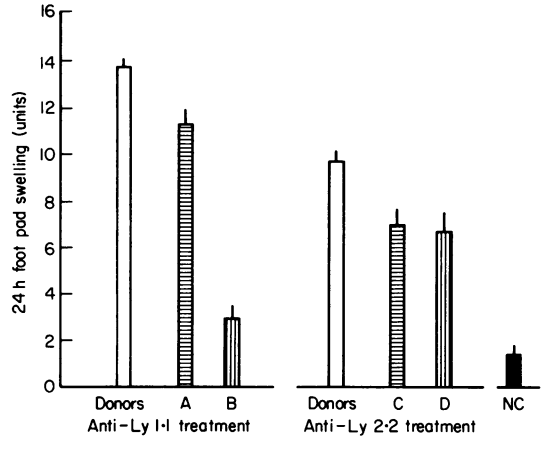

Figure 6. Sensitivity of DTH transferring PEL to anti-Ly 1·1 serum. PEC were collected from CBA or C57Bl/6 donors sensitized by the i.v. injection of 10^8 SRBC under Cy-modulation, 5 days before exudate induction. Nylon wool non-adherent PEC were either treated with complement alone (groups A and C), treated with anti-Ly 1·1 serum plus complement (CBA, group B), or with anti-Ly 2·2 serum plus complement (C57Bl/6, group D). Cells (3×10^6 cells per mouse) were transferred into syngeneic recipients. Five recipients per group ± SE. NC, recipients of nylon wool non-adherent PEL from non-sensitized donors.

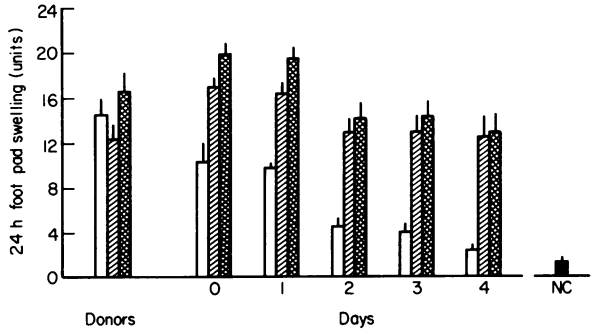

Figure 7. Longevity of DTH transferring PEL in normal recipient mice. Donors had been sensitized with 10^8 SRBC i.v., either under Cy-modulation (open bars), under BCG-modulation (hatched bars), or under modulation by BCG plus Cy (cross-hatched bars). 3×10^7 cells per recipient were transferred and DTH reactions elicited on the days indicated. NC, DTH in recipients of PEC from non-sensitized donors. Groups of five mice each ± SE.

immune modulating agent (Lagrange & Mackaness, 1975). In order to determine whether this is a reflection of different life spans of the T cells involved, one of three pools of normal recipient mice was injected with 3×10^7 PEC from donors sensitized under Cy-modulation, the second pool receiving 3×10^7 PEC from donors sensitized under BCG-modulation, and the third pool receiving 3×10^7 PEC from donors sensitized under the combined influence of BCG plus Cy. DTH reactions were elicited daily in groups of five recipients from each pool and measured 24 h later. DTH rapidly disappeared in recipients of cells from Cy-modulated donors, whereas cells from mice modulated by BCG alone or by BCG plus Cy were able to mediate DTH reactions for a longer period (Fig. 7). Thus, effector T lymphocytes from BCG-treated donors differ from those of mice not pretreated with BCG with respect to their longevity in the circulation of normal mice. As will be demonstrated in the following experiment, cells from BCG-treated mice also show a different susceptibility to the activity of suppressive factors in anergic mice.

Performance of SRBC-specific PEL in anergic mice

Anergic recipient mice were produced as described in materials and methods and divided into two groups: one group received two equivalents of PEC from Cy-modulated immunized donors. The other group received two equivalents of PEC from donors immunized under BCG-plus-Cy-modulation. Immedia-

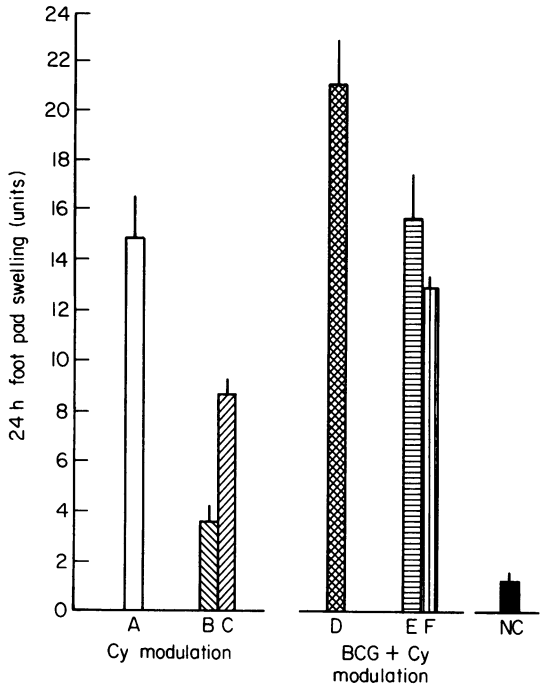

Figure 8. Differential expression of DTH by PEL from Cy-modulated and from BCG-plus-Cy-modulated donors in anergic mice. A, DTH in donors sensitized i.v. with 10^8 SRBC under cy-modulation; B and C, DTH in anergic and normal recipients, respectively, of 2 equivalents of PEC from Cy-modulated donors; D, DTH in donors sensitized with 10^8 SRBC i.v. under BCG-plus-Cy-modulation; E and F, DTH in anergic and normal recipients, respectively, of 2 equivalents of PEC from BCG-plus-Cy-modulated donors. NC, DTH in recipients of 2 equivalents of PEC from normal donors. Groups of five mice each ± SE.

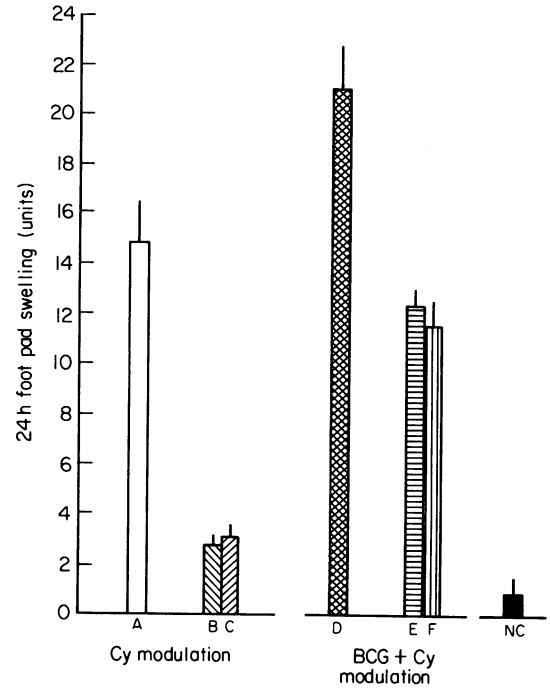

Figure 9. Differential expression of DTH by PEC from mice sensitized under Cy or BCG-plus-Cy-modulation in secondary recipients, after having been 'filtered' through either normal or anergic intermediate recipients. Left hand panel: A, DTH in donors sensitized with 10^8 SRBC i.v. under cy-modulation; B and C, DTH expressed in secondary recipients after passage of PEC through anergic and normal intermediate recipients, respectively. Right hand panel: D, DTH in donors sensitized with 10^8 SRBC under BCG-plus-Cy-modulation; E and F, DTH expressed in secondary recipients after passage of PEC through anergic and normal intermediate recipients, respectively. NC, DTH in recipients of PEC from normal donors. Groups of five animals each ± SE.

tely after transfer, DTH reactions were elicited in both recipient groups.

As seen in Fig. 8, PEL from Cy-modulated donors were unable to confer DTH upon anergic recipients, whereas PEL from BCG-plus-Cy-modulated donors were able to do so. This observation indicates that in anergic mice, a T-cell suppressing principle is active which, however, fails to suppress the expression of DTH by SRBC-specific PEL from BCG-plus-Cy-modulated donors. The lack of susceptibility of cells from BCG-treated donors to the suppressive principle active in anergic mice is in keeping with findings of Lagrange & Mackaness (1975) on the performance of SRBC-specific spleen cells.

Finally, in another type of experiment, PEC from the two donor types were compared with respect to their ability to transfer DTH through intermediate recipients into a second set of recipient mice. In the experiment depicted in Fig. 9, PEC had been transferred (at a 2 : 1 donor/recipient ratio) into intermediate recipients. In the latter, exudates were induced immediately after transferring cells, exudate cells being collected 24 h later for secondary transfer into final recipients (2 : 1 ratio). Intermediate recipients were either normal or anergic mice. As can be seen in Fig. 9, reactive cells from BCG-plus-Cy-modulated donors accumulated in peritoneal exudates of intermediate recipients so as to be able to transfer DTH reactions to a second set of recipients regardless of whether intermediate recipients were anergic or not.

On the other hand, PEC from Cy-modulated donors failed to transfer DTH-reactions to secondary recipients after their passage through either intermediate recipient group. Thus, PEL from BCG-plus-Cy-modulated donors are distinguished, (a) by their longer persistence in circulation, and (b) by a lack of susceptibility to suppressive factors operating in anergic mice.

DISCUSSION

Specifically committed T cells, in order to fulfil functions in the periphery, leave the lymphatic tissues and enter inflammatory foci non-specifically. Their propensity to enter inflammatory foci ensures that they reach any tissue sites and set into motion events which lead to the build-up of a cellular defence (Koster & McGregor, 1971; Asherson & Allwood, 1972).

In recent years, subpopulations of T cells have been identified on the basis of antigenic surface markers, receptors, and biological activity (Snell, 1978). Using DTH to SRBC as experimental model, we have asked the question as to which T cell types can be found amongst inflammatory lymphocytes, and what biological activity they possess.

The results of the present study demonstrate that T cells mediating DTH to SRBC enter casein-induced inflammatory exudates from which they can be recovered and assayed in a passive transfer system. DTH-transferring PEL are found only under conditions which cause DTH in donor mice, i.e. if the dose of sensitizing antigen is low (10^6 i.v.), and if exudates are induced at the height of the immune response (Fig. 2). Immune modulating agents, such as Cy, BCG, or both, considerably enhance the DTH transferring capacity of peritoneal exudates, in parallel with enhancing effects these agents have on the cellular immune response to SRBC (Lagrange & Mackaness, 1975) (Figs 3 and 4).

DTH transferring PEL are T cells as indicated (a) by the sensitivity of DTH transfer to anti-Thy 1·2 serum (Fig. 5), and (b) by the enrichment of DTH transferring cells achieved by nylon wool incubation. Furthermore, they are Ly 1^+, $2,3^-$ (Fig. 6). The same phenotype is expressed by spleen cells mediating DTH to SRBC (Huber, Devinsky, Gershon & Cantor, 1976), and hence this phenotype is preserved after committed T cells have entered inflammatory exudates (Fig. 6).

The duration of DTH-mediating PEL from non-modulated or Cy-modulated mice in circulation after transfer is limited, i.e. in the order of 2 days (Fig. 7). Likewise, transferred PEL from such donors cannot be recovered from exudates of adoptively sensitized intermediate recipients (Fig. 9). In contrast, PEL from BCG-modulated or from BCG-plus-Cy-modulated donors show a much greater longevity in the circulation of recipients (Fig. 7). Cells from BCG-plus-Cy-modulated donors can be recovered from exudates in intermediate recipients and be used to transfer DTH to a second set of recipients (Fig. 9). Moreover, cells from BCG-plus-Cy-modulated donors are not suppressed in anergic recipients (Fig. 8). This indicates that differences exist in the longevity of peritoneal exudate T cells and their susceptibility to suppressor mechanisms, depending on whether cell donors have been subject to immune modulation by BCG, or not. Moreover, peritoneal exudate T cells from Cy-modulated donors are helper cells, whereas cells from BCG-plus-Cy-modulated donors are inactive as helper cells (Hahn, Kaufmann, Falkenberg, Chahin & Horn, 1979).

Based on these findings, the possibility must be entertained that during an immune response to SRBC, depending on the mode of sensitization, different subsets of T cells may be induced or different stages of differentiation reached: Without mycobacterial modulation, T cells are formed which mediate an evanescent form of DTH (Jones-Mote type), and T helper cells occur, whereas under mycobacterial modulation, T cells are produced which mediate tuberculin-like hypersensitivity, but are not helper cells.

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REFERENCES

ASHERSON G.L. & ALLWOOD G.G. (1972) Inflammatory lymphoid cells. Cells in immunized lymph nodes that move to sites of inflammation. *Immunology*, **22**, 493.

ASHERSON G.L., ALLWOOD G.G. & MAYHEW B. (1973) Contact sensitivity in the mouse. Movement of T blasts in the draining lymph nodes to sites of inflammation. *Immunology*, **25**, 485.

HAHN H., KAUFMANN S.H.E., FALKENBERG F., CHAHIN MARYAM & HORN W. (1979) Peritoneal exudate 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. *Immunology*, (In press.)

HUBER B., DEVINSKY O., GERSHON R.K. & CANTOR H. (1976) Cell-mediated immunity: delayed-type hypersensitivity and cytotoxic responses are mediated by different T-cell subclasses. *J. exp. Med.* **143**, 1534.

JULIUS M.H., SIMPSON E. & HERZENBERG L.A. (1973) A rapid method for the isolation of functional thymus derived murine lymphocytes. *Europ J. Immunol.* **3**, 645.

KOSTER F.T. & MCGREGOR D.D. (1971). The mediator of cellular immunity. III. Lymphocyte traffic from the blood into the inflamed peritoneal cavity. *J. exp. Med.* **133**, 864.

LAGRANGE P.H., MACKANESS G.B. & MILLER T.E. (1974) Influence of dose and route of injection on the immunological induction of T cells. *J. exp. Med.* **139**, 528.

LAGRANGE P.H. & MACKANESS G.B. (1975) A stable form of delayed-type hypersensitivity. *J. exp. Med.* **141**, 82.

LANDSTEINER K. & CHASE M.W. (1942) Experiments on transfer of cutaneous sensitivity to simple chemical compounds. *Proc. Soc. Exp. Biol.* **49**, 688.

MILLER T.E., MACKANESS G.B. & LAGRANGE P.H. (1973) Immunopotentiation of the response to sheep red blood cells. *J. nat. Cancer Inst.* **51**, 1669.

NORTH R.J. & SPITALNY G. (1974) Inflammatory lymphocytes in cell-mediated antibacterial immunity: factors governing the accumulation of mediator T cells in peritoneal exudates. *Infect. Immunity*, **10**, 489.

SHEN F.W., BOYSE E.A. & CANTOR H. (1975) Preparation and use of Ly antisera. *Immunogenetics*, **2**, 591.

SNELL G.D. (1978) T cells, T cell recognition structures, and the major histocompatibility complex. *Immunol. Rev.* **38**, 3.