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# Delayed-type hypersensitivity and allograft rejection in the mouse: correlation of effector cell phenotype

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**Summary.** Delayed-type hypersensitivity (DTH) responses to alloantigens were found to correlate with both skin and tumour allograft rejection in 224 reconstituted ATXBM-CBA mice. Furthermore, DTH responses and allograft rejection were observed only in mice that had received Ly-1 cells. Depletion of Thy-$1^+$ or Ly-$1^+$ cells led to indefinite graft survival and the absence of DTH responses, whereas depletion of Ly-$2^+$ cells led to rapid graft rejection and strong DTH responses. The same result was obtained with CBA mice responding to grafts of either C57BL/6 skin, the B16 melanoma, or the EL4 lymphoma; and for (CBA × A)$F_1$ mice responding to *H-2K* region alloantigens of AQR skin grafts. Thus, DTH and allograft rejection are both mediated by a Ly-1 T cell and it is considered that these are two different manifestations of the same transplantation response.

## INTRODUCTION

The mechanisms of allograft rejection are complex and involve a number of interacting factors of which cellular immune mechanisms are predominant in the mouse. Graft rejection is readily understood in terms of specific killer T cells causing allograft destruction, however this concept is not well founded as there is limited recovery of cytotoxic T cells from rejecting tissues (Roberts & Hayry, 1977; Ascher, Ferguson, Hoffman & Simmons, 1979), and furthermore, this concept is in conflict with our previous findings (Loveland, Hogarth, Ceredig & McKenzie, 1981; Loveland & McKenzie, 1982). Thus Ly-1 cells are required for the induction and expression of skin and tumour allograft rejection whereas cytotoxic (Ly-123) T cells appear to play little if any role in rejection. In addition, mice repleted with Ly-1 cells and which lacked detectable Ly-123 cells (the Ly-2-depleted ATXBM mice) rapidly rejected grafts.

In search of the link between these Ly-1 effector cells and the mechanisms of graft rejection, two questions arise: (i) what populations of cells are capable of tissue destruction, and (ii) how can Ly-1 cells regulate the process of tissue destruction? Ly-1 cells generally appear to function by interacting with other cells of the immune repertoire and are not normally considered to be cytotoxic cells. Traditional concepts have linked delayed-type hypersensitivity (DTH) responses and transplantation immunity (Brent, Brown & Medawar, 1958; 1962) because of their joint induction, adoptive transfer, and long term persistence. In addition, the T-cell-dependent DTH response is understood to involve a complex interaction of T and B lymphocytes, antibodies, macrophages, lymphokines and a predominantly monocytic infiltrate (Crowle, 1975). In

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order to examine this implied relationship, ATXBM mice reconstituted with Ly-1 cells (Ly-2-depleted), Ly-2 cells (Ly-1-depleted), mixtures of these populations (lacking Ly-123 cells), or undepleted cells (Ly-1, Ly-23 and Ly-123 cells), were assayed for their DTH responsiveness to alloantigens in the foot-pad swelling assay. We report here the correlation of DTH responses and graft rejection which strongly suggests a relationship between these phenomena.

## MATERIALS AND METHODS

*Mice*

Mice were bred and maintained at the Austin Hospital. Male mice of the following strains were used: CBA/H ($H\text{-}2^k$); C57BL/6J ($H\text{-}2^b$); (CBA × B6)$F_1$ ($H\text{-}2^{k/b}$); (CBA × A)$F_1$ ($H\text{-}2^{k/d}$); and AQR/Sf ($H\text{-}2^q$). Adult thymectomized, irradiated, bone marrow reconstituted (ATXBM) mice were prepared as described elsewhere (Loveland *et al.*, 1981).

*Antibodies*

The preparation and characterization of the monoclonal antibodies to the Ly-1·1 and Ly-2·1 specificities have been described (Hogarth, Potter, Cornell, McLachlan & McKenzie, 1980; Hogarth, Edwards, McKenzie, Goding & Liew, 1982). Pools of tissue culture supernatant were selected for strong complement-dependent cytotoxicity. Alloantisera to the Thy-1·2 specificity were made in the congenic combination (B6PL(74NS) × RF)$F_1$ anti-C57BL/6.

*Lymphocyte suspensions and reconstitution of ATXBM-CBA mice*

Lymphoid cell suspensions were prepared from the spleen and lymph nodes of naive (non-sensitized) or sensitized syngeneic donors. Mice were sensitized by one or more skin grafts and an intraperitoneal (i.p.) dose of $2 \times 10^7$ lymphoid cells, the last being 2–3 weeks before their use as donors. T-cell suspensions were depleted by a double treatment with antibody and rabbit complement before adoptive transfer to ATXBM recipients. Thus, 'undepleted' mice received Ly-1, Ly-123 and Ly-23 T cells, whereas 'Ly-1-depleted' mice received Ly-23 T cells, 'Ly-2-depleted' mice received Ly-1 T cells, and 'Thy-1·2-depleted' mice received no T cells. Depletions were complete as has been shown previously (Loveland *et al.*, 1981).

*Skin grafts*

Allogeneic skin grafts were transplanted to ATXBM mice one day after the adoptive transfer of lymphoid cells (Billingham & Medawar, 1957).

*Maintenance and use of tumours*

The two C57BL/6 (H-$2^b$) tumours, B16 melanoma and EL4 lymphoma, were maintained in the subcutaneous and ascites forms, respectively, in (CBA × B6)$F_1$ mice. For experimental use, single cell suspensions were injected subcutaneously (s.c.) in 0·10 ml using a 30 G needle. The perpendicular diameters of the subsequent tumours were measured with calliper squares and recorded as the mean tumour diameter. This parameter was proportional to the volume of the tumour.

*DTH assay*

The delayed-type hypersensitivity (DTH) response to allogeneic cells was assayed by measurement of the specific footpad swelling (Gray & Jennings, 1955). When used, cyclophosphamide (Endoxan-Asta) at 100 mg/kg was injected i.p. 2 days before sensitization. Mice were sensitized either by a skin graft within 21 days of challenge or by the s.c. injection of $3 \times 10^7$ lymphoid cells 6 days before challenge. They were challenged with $5 \times 10^6$ or $1 \times 10^7$ lymphoid cells in 0·04 ml volumes injected via a 30 G needle into one hind footpad and medium was injected into the other footpad. Both footpads were measured to ±0·03 mm before injection and at 24 hr with a Schnelltaster dial gauge calliper (Diatest Ltd, London) and means of these measurements were used to calculate the percent specific swelling.

*Treatment of data*

Means and standard errors were calculated using a programmable Hewlett Packard 25C calculator and statistical significance was determined by Student's *t* test.

## RESULTS

### Time course of the DTH response in ATXBM mice

ATXBM-CBA mice reconstituted with $1 \times 10^6$ lymphoid cells obtained from sensitized donors rejected a subsequent C57BL/6 skin graft (MST 12·7 ± 0·8 days). They were then boosted with (CBA × B6)$F_1$ cells on day 18 and six days later were challenged with (CBA × B6)$F_1$ cells injected into one hind footpad. After 24 hr, an increase in mean footpad thickness of 0·27 ± 0·02 mm (17% ± 1%) was measured, a swelling that reduced within 48 hr (mean swelling 0·11 ± 0·05

mm; 7%±3%)—Fig. 1. Normal mice sensitized and challenged by the same protocol exhibited similar footpad swelling (0·40±0·06 mm; 20%±4%, reducing to 0·17±0·05 mm; 11%±3%) whereas normal mice that only received a footpad injection produced

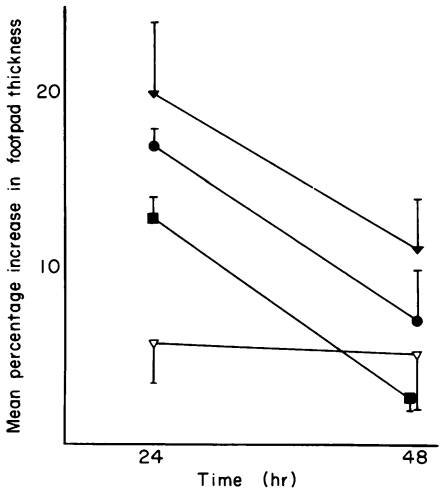


**Figure 1.** Time course of the DTH response in ATXBM-CBA mice measured in hours as the mean percentage increase in footpad thickness. The ATXBM-CBA recipients had been reconstituted with either $1 \times 10^6$ sensitized CBA lymphocytes (●) or $3 \times 10^6$ sensitized, Ly-2-depleted lymphocytes (■). Control groups were normal CBA mice sensitized and challenged as above (▼), or challenged only (▽). There were four mice per group.

'non-specific' swelling of 0·10±0·03 mm (6%±2%) after 24 hr and 0·08±0·04 mm (5%±3%) after 48 hr. In the same experiment (Fig. 1), a group of ATXBM-CBA mice reconstituted with $3 \times 10^6$ sensitized Ly-2-depleted lymphocytes exhibited a DTH response. The specific swelling was 0·22±0·02 mm (13%±1%) at 24 hr and reduced to 0·04±0·02 mm (2·5%±1%) at 48 hr. Thus, the DTH response in reconstituted ATXBM-CBA mice was similar to that elicited in normal sensitized mice and could be assayed in Ly-2-depleted ATXBM mice, evidence of an Ly-1 cell being the mediator of DTH.

### Cyclophosphamide pretreatment and the DTH response

The DTH response to alloantigens is generally elicited in mice after pretreatment with cyclophosphamide (C-Y) (Smith & Miller, 1979). Sensitized, C-Y-pretreated mice (not ATXBM) developed specific footpad swelling of 49%±5% whereas mice sensitized but not treated with C-Y developed specific swelling of 29%±4% (Fig. 2). Non-specific swelling of 16%–18%, due to the high challenge dose in this experiment of $1{\cdot}5 \times 10^7$ lymphoid cells, was unaffected by treatment of the responders with C-Y. By contrast, ATXBM-CBA mice which had been reconstituted with $4 \times 10^6$ sensitized lymphocytes and had rejected a C57BL/6 skin graft at least 3 weeks earlier, developed DTH responses which were not augmented by pretreatment with C-Y (32% and 34% specific swelling)—Fig. 2.

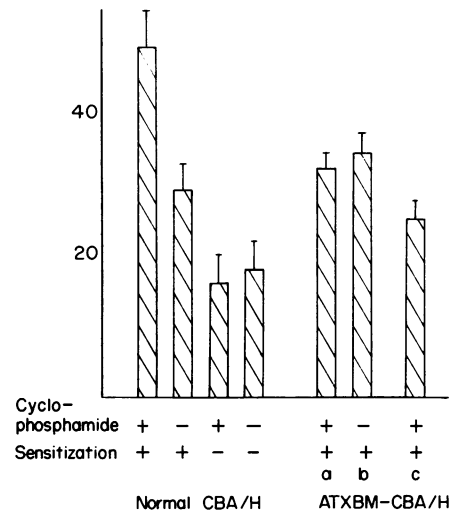


**Figure 2.** Cyclophosphamide pretreatment and the DTH response in normal and ATXBM-CBA mice. Groups of four–six ATXBM-CBA mice had been reconstituted with sensitized syngeneic lymphocytes (a and b, $4 \times 10^6$ cells; c, $3 \times 10^6$ Ly-2-depleted cells) and given a C57BL/6 skin graft 3 weeks before the DTH assay.

ATXBM mice given Ly-2-depleted (Ly-1) cells were also capable of the DTH response (24%±2% swelling) with no evidence of augmentation by C-Y. Thus, in the following experiments C-Y was not used because it did not augment the DTH response in reconstituted ATXBM mice and also the ATXBM mice were physically weakened by C-Y treatment.

### The phenotype of the DTH effector cell

ATXBM-CBA mice which had been both reconstituted with antibody and complement-treated lymphoid cell suspensions and challenged with a C57BL/6 skin graft more than 120 days previously, were assayed

for DTH responses. All the mice were in good health and had either rejected the skin grafts rapidly (MST 14–16 days), in a very delayed manner (> 55 days), or not at all at the time of assay. Mice reconstituted with untreated lymphocytes responded strongly to allogeneic challenge in the DTH assay (mean swelling 44%±6%)—Fig. 3. There was no DTH response elicited in either Thy-1-depleted mice (6%±5% swelling) or in Ly-1-depleted mice (6%±2%, and 12%±5% with delayed skin graft rejection). Furthermore, reconstitution with Ly-2-depleted cells led to rapid skin graft rejection (MST 15·5±1·0 days) and a strong DTH response (35%±4% swelling). It was concluded that Ly-1 cells alone were required to elicit the DTH response because the mixing of the two Ly-depleted populations at reconstitution (group F, Fig. 3) enabled a strong footpad swelling reaction (23%±7%; $P > 0.15$) and the mixture of Ly-1 and Ly-23 cells was, in effect, the same as the Ly-1 cells in eliciting DTH responses, i.e. the absence of Ly-123 cells was without effect. The results clearly demonstrate strong DTH responses (mean swelling > 23%) or no DTH response (mean swelling < 12%) and identify a long lived, adoptively transferred T cell responsible for the DTH phenomenon which has the phenotype Thy-1$^+$ Ly-1$^+$2$^-$. The correlation of the DTH responses and skin graft rejection should be noted. Of the sixty-seven mice detailed in Fig. 3, fifty-one that had rejected grafts had measurable DTH responses, whereas the sixteen that did not reject skin grafts had no discernible DTH response. Furthermore, in the group with slow (weak) rejection, (group D, Fig. 3), there were correspondingly very weak, but discernible, footpad swelling reactions.

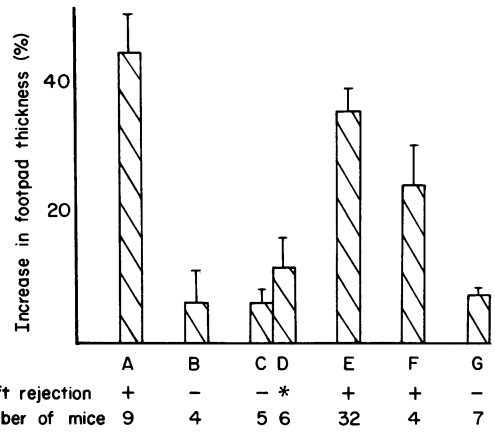


**Figure 3.** DTH responses in reconstituted ATXBM-CBA mice. The reconstituting lymphocyte doses were $1 \times 10^6$ untreated cells (A); $1 \times 10^6$ Thy-1·2-depleted cells (B); $2 \times 10^6$ Ly-1-depleted cells (C,D); $2 \times 10^6$ Ly-2-depleted cells (E); the mixture of C and E (F); and no cells (G). Skin grafts were rejected rapidly (MST 14–16 days) (+), after more than 55 days (*), or were healthy at the time of assay (120–150 days) (—).

## The correlation of DTH responsiveness with tumour allograft rejection

To determine whether the transplantation responses generated against different tissues correlated with DTH responses, reconstituted ATXBM mice were injected with B16 melanoma or EL4 lymphoma cells and assayed for footpad swelling before progressive tumours killed the susceptible mice. ATXBM-CBA mice reconstituted with sensitized lymphocytes and injected s.c. with $1 \times 10^7$ EL4 cells rejected the tumour and exhibited strong DTH responses (mean swelling 18·5%±2%)—Fig. 4. However, no DTH responses were observed in Ly-1-depleted (mean swelling 1%±0·7%) or untreated ATXBM mice (mean swelling 3%±1%), all of which did not reject the tumour and subsequently died within 4 weeks. Ly-2-depleted ATXBM mice rejected the tumour and developed moderate swelling (9%±1%), significantly more than the ATXBM controls ($P < 0.05$). Therefore, both tumour rejection and the induction of DTH responsiveness corresponded with the presence of Ly-1 cells in the responding mice.

Similarly, variously reconstituted ATXBM mice were injected with B16 melanoma cells and challenged by the footpad assay 2 weeks later. As shown in Fig. 5, mice reconstituted with untreated lymphoid cells or Ly-2-depleted cells rejected the tumour allograft and developed strong DTH responses (groups A, B, E and F). ATXBM mice (group G) and Ly-1-depleted mice (groups C and D) had progressively growing tumours and did not exhibit specific footpad swelling (< 6%). These mice were in good health, at this stage unaffected by the tumour, and the lack of the DTH response could not be attributed to any physical weakness. Two control groups were included in these experiments, one of sensitized normal CBA mice (group I) as a positive control (16%±3% mean swelling) and one of naive normal CBA mice (group H) as a negative control (5%±2·5% mean swelling). Hence, the positive and negative responses obtained in

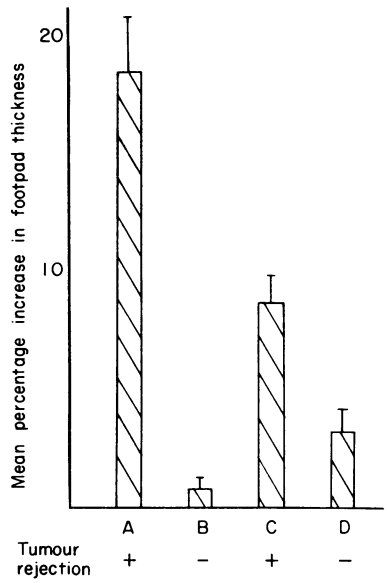


**Figure 4.** DTH responses in reconstituted ATXBM-CBA mice grafted with the EL4 lymphoma. ATXBM-CBA mice were reconstituted with syngeneic sensitized lymphocytes and after 3 weeks were injected s.c. with $1 \times 10^7$ allogeneic EL4 cells. Tumours either grew progressively (groups B and D) or were rejected (A, $11{\cdot}1 \pm 0{\cdot}4$ days; C, $10{\cdot}5 \pm 0{\cdot}3$ days) according to the reconstituting inoculum (A: $1{\cdot}5 \pm 10^6$ untreated cells; B: $1{\cdot}5 \times 10^6$ Ly-1-depleted cells; C: $1 \times 10^6$ Ly-2-depleted cells; D: no cells). The DTH response was assayed 18 days after EL4 injection in groups of five–seven mice.

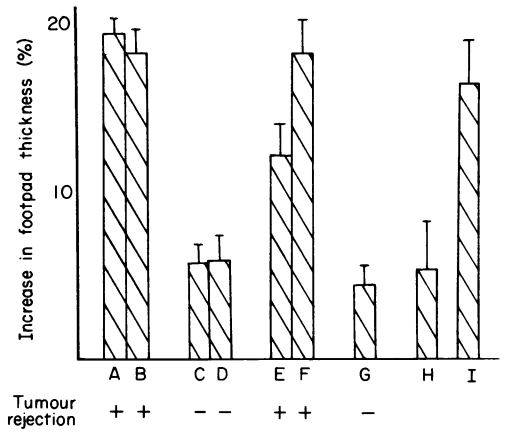


**Figure 5.** DTH responses in reconstituted ATXBM-CBA mice grafted with the B16 melanoma. ATXBM-CBA mice were reconstituted with syngeneic lymphocytes (A: $2 \times 10^6$ untreated; B: $4{\cdot}5 \times 10^6$ untreated; C: $1 \times 10^6$ Ly-1-depleted; D: $2 \times 10^6$ Ly-1-depleted; E: $1{\cdot}5 \times 10^6$ Ly-2-depleted; F: $3 \times 10^6$ Ly-2-depleted; G: no cells) and after 2 weeks were injected s.c. with $3 \times 16^6$ allogeneic B 16 cells. DTH responses were elicited 2 weeks later. Controls were naive CBA mice (group H) or sensitized CBA mice (group I). Two independent experiments are represented with four–ten mice per group.

ATXBM mice were consistent with those of normal mice.

In summary, both the EL4 lymphoma and B16 melanoma induced DTH responses which correlated completely with tumour rejection and the presence of Ly-1 effector T cells.

### DTH responses against *H-2K* alloantigens

The experiments described above support the hypothesis that both allograft rejection and the DTH response are mediated by Ly-1 cells and that conditions which lead to one response generate effector cells of the other. The transplantation responses generated to the restricted differences of H-2K antigens also identified an Ly-1 effector cell for skin graft rejection (unpublished data) and in the following experiments the DTH response was examined.

In Fig. 6, DTH responses of $(CBA \times A)F_1$ mice to AQR/Sf strain antigens ($H\text{-}2K^q$) are depicted.

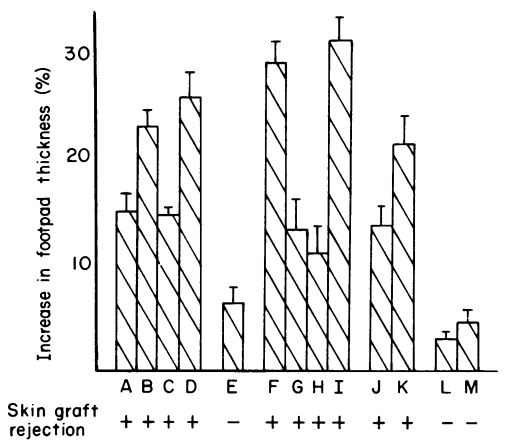


**Figure 6.** DTH responses to H-2K alloantigens. ATXBM-$(CBA \times A)F_1$ mice were reconstituted with syngeneic cells and given an AQR graft 20 to 50 days before the DTH assay. Groups of five–nine mice were reconstituted with untreated lymphoid cells (A: $3 \times 10^5$; B: $1 \times 10^6$; C: $2 \times 10^6$; D: $8 \times 10^6$), Ly-1-depleted cells (E: $5 \times 10^5$), Ly-2-depleted cells (F: $2 \times 10^5$; G: $7 \times 10^5$; H: $1{\cdot}5 \times 10^6$; I: $3 \times 10^6$), or mixtures of the depleted populations (J: $5 \times 10^5$ Ly-$1^-$/$3 \times 10^5$ Ly-$2^-$; and K: $5 \times 10^5$ Ly-$1^-$/$1 \times 10^6$ Ly-$2^-$). Controls were ATXBM mice with surviving allogeneic skin grafts (L,M). DTH responses were elicited to AQR spleen cells.

ATXBM mice reconstituted with various doses of untreated lymphoid cells and having already rejected skin grafts, exhibited footpad swelling (15%–26%, groups A–D). By contrast, Ly-1-depleted mice neither rejected skin grafts nor generated DTH responses (group E), which can be taken as evidence of a Ly-1$^+$ effector T cell involved in both responses. Ly-2-depleted mice exhibited moderate to strong footpad swelling (11%–31%, groups F–I). This variation appeared unrelated to the reconstituting cell dose, the mean skin graft survival time, or the particular experiment (three are represented). The mixing of the depleted cell populations at reconstitution led to both graft rejection and DTH responses (14%–21% mean swelling), equivalent to the adoptive transfer of Ly-1 cells alone. As a control group, ATXBM mice neither rejected skin grafts nor generated DTH responses (3%–5% swelling; groups L and M). These findings confirm the central role of Ly-1 cells in transplantation responses restricted to *H-2K* region antigens.

## DISCUSSION

The generation and expression of the DTH response in mice was shown to require Ly-1 cells in immune responses generated to multiple H-2 and non-H-2 antigenic differences in both skin and tumour allografts, and to restricted H-2K antigenic differences for skin grafts. There were no differences observed in the induction and expression of DTH by any of these alloantigenic stimuli. In a total of 182 ATXBM mice reconstituted with lymphoid cell suspensions containing Ly-1 cells, all animals rejected either skin or tumour allografts and exhibited DTH responses. Furthermore, a total of 42 Ly-1-depleted mice did not produce footpad swelling greater than non-specific levels and either did not reject grafts or did so in a chronic manner after a long delay ($>55$ days). No mice were found that rejected grafts rapidly and failed to produce a DTH response or produced a DTH response while bearing an intact skin or tumour graft. Therefore, in 224 mice, a consistent relationship between graft rejection and the DTH response was established. The strong DTH responses of Ly-2-depleted mice showed that the DTH phenomenon was generated from Ly-1-precursor cells and mediated by Ly-1 effector cells, a further correlation with skin graft rejection.

The impact of these studies is that they relate DTH responses to the rejection of both skin and different tumour cells at the level of the effector cell and the only T cell required for the expression of these two responses is the Ly-1 cell (Loveland *et al.*, 1981; Loveland & McKenzie, 1982). It should be noted that the Ly-123 cell has no functional role in these phenomena because depletion by the Ly-2 antibody was without effect. Hence, the current concepts of killer T cells causing graft rejection are inadequate to explain the models of rejection presented herein. The subsequent discussion is focused on two questions: how Ly-1 cells generate the mechanisms of tissue destruction and whether one Ly-1 cell is responsible for both phenomena.

From the studies presented herein, it is concluded that the induction of both DTH and graft rejection occurs by the activation of specific Ly-1 cells that are responsible for the recognition of alloantigens, and the generation of the subsequent immune responses. In their absence, there is no generation of alloreactivity, and other cells such as macrophages, mast cells and killer T cells, are not independently activated, as observed *in vivo* in Ly-1-depleted ATXBM mice in which allografts were not rejected and no DTH responses occurred. Furthermore, the finding that Ly-1-depleted, *sensitized* lymphoid cells were immunoincompetent suggested that Ly-1 cells were required to maintain the previously induced transplantation response.

DTH responses to transplantation antigens are generally assayed in direct reactions (e.g. in these studies as a host *versus* graft response) and also in the GVH reactions of the immune lymphocyte transfer test and the normal lymphocyte transfer test (Brent & Medawar, 1966; Streilein & Billingham, 1970). The histological similarities between these DTH responses (Turk, 1967; Crowle, 1975) and the course of skin graft rejection (Billingham, Brent & Medawar, 1954; Eichwald, Wetzel & Lustgraaf, 1966; Eman & Eichwald, 1981) are found in the predominance of mononuclear cells (both lymphocytes and macrophages), the progressive changes of the cellular constituents in the infiltrate from lymphocytic to phagocytic, and the subsequent necrotic changes in the dermis and epidermis. The definition of T cells taking part in these responses is not precise because of the limited techniques to identify them in tissue sections and the difficulties of isolating them from the tissue. Thus, cytotoxic T cells are identified among the infiltrating cells in graft rejection although in the latter stages of rejection, most cytotoxicity is reported to be mediated

by non-T cells (Wiktorowicz, Roberts & Hayry, 1978; Ascher *et al.*, 1979).

The production of lymphokines by Ly-1 cells is the probable mode by which Ly-1 cells exert their effects and is the method of modulating the functions of other cells without the need for direct cell apposition, although at present, the *in vivo* role of lymphokines is still to be proven. In this context it should be noted that costimulator (interleukin 2(IL-2)), a product of concanavalin A (Con A)-stimulated Ly-1 cells in the presence of macrophages, is necessary for the mitogenic stimulation of thymocytes and enhances both the level of DNA synthesis in the MLR and the generation of CTL *in vitro* (Shaw, Monticone, Mills & Paetkau, 1978; Shaw, Pilarski, Al-Aldra, Leigh, Wilkins, Hogarth, McKenzie & Paetkau, 1981). Purified interleukin 2, in functions distinct from interferon, can also augment natural killer (NK) cell activity (Henney, Kuribayashi, Kern & Gillis, 1981). Other cells influenced by factors produced by Ly-1 T cells include mast cells (Nabel, Galli, Dvorak, Dvorak & Cantor, 1981), a most significant observation in the light of mast cell involvement in hypersensitivity responses. In addition, a recent report describes the ability of lymphocytes taken from recipients of rejecting allografts to modulate macrophage production of plasminogen activator, a substance which gives activated macrophages a fibrinolytic capacity and may enable them to invade allografts (Tilney, Fleming & McLoughlin, 1981). There is therefore ample additional evidence of the role of Ly-1 T cells in transplantation responses.

Findings pertinent to this model of rejection have been made in other systems. In the rat, the monoclonal antibodies produced against the W3/25 and MRC OX8 specificities distinguish helper T cells, T cells involved in the MLR and GVH responses (W3/25$^+$ MRC OX8$^-$) from suppressor T cells (W3/25$^-$ MRC OX8$^+$) (Brideau, Carter, McMaster, Mason & Williams, 1980; White, Mason, Williams, Galfre & Milstein, 1978). These functional groupings may be of particular significance in associating helper T cells and GVH effector cells, similar to the helper cell—graft rejection—DTH effector cell Ly-1 phenotype in the mouse. In addition, a mouse monoclonal antibody (OKT4), when administered to monkeys *in vivo*, has led to lengthened renal allograft survival (Cosimi, Burton, Kung, Colvin, Goldstein, Lifter, Rhodes & Russell, 1981). This antibody is specific for the helper-inducer T-cell subset in humans and not the cytotoxic T cells, which is indirect evidence for the 'helper' T cell to be important in graft rejection in man and this is also consistent with our findings.

The inter-relationship of DTH with graft rejection is a potential clinical tool for assessment of the immunogenicity of proposed tissue donors, possibly as a skin test in either the recipient or the irradiated-hamster test (Ramseier & Streilein, 1965). The findings presented herein suggest that assay of DTH responsiveness would be a predictive test for transplantation responses in general. Indeed, DNCB skin reactions in renal dialysis patients before kidney transplantation, albeit non-specific but indicative of intact DTH function, have been reported to be of use in clinical prognosis, predicting the likelihood of later rejection crises (Rolley, Sterioff, Parks & Williams, 1977).

In summary, DTH and graft rejection are concluded to be different manifestations of the one transplantation response mechanism. They are related by the concept of a single, specific Ly-1 effector T cell, with the capacity for the initiation of a number of different responses. A corollary of this hypothesis is that Ly-1 helper T cells and the proliferative Ly-1 cell of the MLR may be the same as the Ly-1 cell described herein, and the nature of these responses is compatible with the role of the DTH/graft rejection T cell. If so, a single population of Ly-1 cells would provide 'help' for all interacting cells, including macrophages, mast cells, CTL, NK cells and B cells.

## ACKNOWLEDGMENTS

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