A REEXAMINATION OF THE ROLE OF LYT-2-POSITIVE T CELLS IN MURINE SKIN GRAFT REJECTION

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When it was discovered that a subclass of T lymphocytes could specifically lyse target cells expressing foreign histocompatibility antigens, it was a natural assumption that such cells would be the effector cells in skin allograft rejection. Since that time, a great deal of circumstantial evidence has been presented to implicate cytotoxic T lymphocytes (CTL)[1] in the phenomenon of graft rejection (reviewed in 1). It should be stressed, however, that all of the evidence is circumstantial and that there probably is more than one mechanism to reject an allogeneic skin graft (2, 3). The recent and much publicized work of Loveland, McKenzie, and their colleagues (4–6) directly challenges the notion that CTL are involved in murine skin graft rejection. These workers constructed T cell-deficient mice by thymectomizing adult CBA (H-2^k) mice, then irradiating and reconstituting them with T cell-deficient syngeneic bone marrow. Such ATXBM mice were unable to reject C57BL/6 (H-2^b) skin grafts but an inoculum of primed CBA lymphoid cells restored their competence in this regard. The provocative finding was that depleting the inoculum of Lyt-2^+ cells with a monoclonal antibody plus complement (C′) had no effect on the adoptive transfer of the ability to reject. Lyt-1-specific antibody plus C′ did deplete the effective cells but in this case (CBA mice that express the Lyt-1.1 allele), the anti-Lyt-1 sera killed all T cells.

Although their experimental result seemed clear, in that recipients of antigen-primed Lyt-2-depleted cells did reject skin grafts, it was formally possible that host-derived T cells, including CTL, were also contributing to the response. However, a functional analysis of the adoptive hosts after rejection of the skin grafts was not performed. We realize that it is very difficult to make truly T cell-free mice: nude mice (7–10) as well as ATXBM mice (11) contain T cell precursors including CTL. Therefore, we designed experiments which used a more stringent protocol for T cell depletion of ATXBM hosts and we used alloantigen-primed Thy-1.1 T cells to transfer immunity to congeneic Thy-1.2 ATXBM hosts. This scheme enabled us to distinguish between activity due to the adoptively transferred cells (Thy-1.1) and the host-derived T cells (Thy-1.2). Using minor histocompatibility antigens as a graft barrier, we demonstrated that Lyt-2-depleted as well as Lyt-1-depleted immune spleen cells could transfer

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[1] *Abbreviations used in this paper:* ATXBM, adult thymectomized, irradiated bone marrow-reconstituted mice; C′, complement; Con A, concanavalin A; CTL, cytotoxic T lymphocyte; MAF, macrophage-activating factor; MLC, mixed lymphocyte culture.

immunity to ATXBM hosts that themselves do not reject grafts. However, our results showed that adoptive hosts which have received anti-Lyt-2 plus C′-treated immune spleen cells and have rejected skin grafts contain graft-specific CTL of host origin. Furthermore, when ATXBM mice are skin grafted while the host T cell response is absent, donor-derived CTL are detected even in animals that received anti-Lyt-2 plus C′-treated immune cells. We conclude that the question whether CTL are the effector cells in graft rejection is still open, but we maintain that the experiments of Loveland et al. (4) do not provide any strong evidence against their involvement.

Materials and Methods

Mice. C57BL/6J (H-2^b, Thy-1.2; designated here as B6.Thy-1.2) were obtained from the vivarium at Scripps Clinic and Research Foundation. BALB.B (H-2^b) mice and C57BL/Ka (H-2^b, Thy-1.1 [B6.Thy-1.1]) were obtained from I. L. Weissman, Department of Pathology, Stanford University Medical Center Stanford, CA.

Monoclonal Antibodies. Monoclonal antibodies used for cell depletions were: 13-4, anti-Thy-1.2 (12); 22.1, anti-Thy-1.1 (12); T24/31.7 (T24) from a rat-mouse hybridoma that recognizes a mouse-specific determinant on Thy-1 (13); AD4(15), anti-Lyt-2.2 (14); and C3PO, anti-Lyt-1.2 (15). All antibodies were in the form of high titer ascites fluid. In our hands, both AD4 (15) and C3PO lyse ~50% of peripheral T cells from B10 mice (14, 15). These are partially overlapping populations since ~16% of peripheral T cells are sensitive to both antibodies. In functional tests on in vivo primed, mixed lymphocyte culture (MLC), boosted anti-minor CTL effectors, we find that >95% of such CTL are sensitive to anti-Lyt-2.2 plus C′ whereas anti-Lyt-1.2 plus C′ inactivates <20% of effector CTL. Additionally, when primed spleen cells are treated before culture, anti-Lyt-2 plus C′ treatment prevents the development of >98% of specific cytotoxicity while treatment with anti-Thy-1 plus C′ removes all CTL activity. C′ treatment alone or treatment with anti-Lyt-1 plus C′ did not affect the CTL response.

ATXBM Mice. 2 d before use as bone marrow donors, B6.Thy-1.2 mice were given a single intraperitoneal injection of 200 μl of rabbit anti-mouse thymocyte serum (Microbiological Associates, Bethesda, MD). Bone marrow cells from tibias and femurs were depleted of Thy-1^+ cells by treatment with a mixture of the 13-4 and T24 monoclonal antibodies at 4°C for 30 min at a cell concentration of 1.0×10^7/ml in Hank's balanced salt solution. Rabbit serum as a source of C′ was then added to adjust the cell concentration to 5.0×10^6 cells/ml and the cells were incubated for 45 min at 37°C, washed twice, and used for reconstitution of syngeneic, thymectomized, irradiated mice. B6.Thy-1.2 mice, 5–7 wk old, were thymectomized and, 1–6 wk later, were lethally irradiated (950 rad) with a ^{137}Cs source and injected intravenously with 1.0×10^7 or 2×10^6 anti-Thy-1 plus C′-treated bone marrow cells. At the time of sacrifice, all ATXBM mice were examined and found to be completely thymectomized.

Priming to Minor Histocompatibility Antigens and Adoptive Transfer. B6.Thy-1.1 mice were primed to minor histocompatibility antigens of the BALB background with a single intraperitoneal injection of 2.0×10^7 viable spleen cells from BALB.B mice. At least 4 wk later, spleen cells were removed and treated with C′ alone or with monoclonal antibodies specific for Thy-1, Lyt-1.2, or Lyt-2.2 plus C′, as described for bone marrow depletion above. After antibody treatments, 5.0×10^6 viable recovered cells were injected intravenously into the ATXBM B6.Thy-1.2 mice on the same day the skin grafting was done.

Skin Grafting. ATXBM B6.Thy-1.2 mice were given a single full thickness BALB.B skin graft (0.6–1.0 cm Diam) on the flank and the dressings were removed 7 d after grafting. Rejection was scored as complete necrosis of the graft.

MLC. For secondary in vitro restimulation, 2.5×10^7 spleen cells from grafted animals were co-cultured with 2.5×10^7 irradiated (1,000 rad from a ^{137}Cs source) BALB.B stimulator cells in 20 ml of RPMI 1640 medium containing 5% fetal calf serum, 10%

supernatant from concanavalin A (Con A)-stimulated mouse spleen cells (16), and 50 mM α-methyl-D-mannoside to block the action of residual Con A. Cultures were incubated for 5 d in an atmosphere of 5% CO_2 in air.

Assay for CTL Activity and Thy-1 Typing. MLC cells were harvested after 5 d, washed, resuspended in Hank's balanced salt solution containing 2 mg/ml bovine serum albumin, and divided into three aliquots. Cells were treated either with C′ alone, 22.1 (anti-Thy-1.1) plus C′, or a mixture of 22.1 and 13.4 (anti-Thy-1.2) plus C′ as described above. After treatment, the cells were washed and assayed in threefold serial dilutions for lysis of ^{51}Cr-labeled target cells.

The concentrations of anti-Thy-1 antibodies and C′ used for typing CTL activity were shown to be sufficient for allele-specific killing in control experiments using in vitro generated CTL from B6.Thy-1.2 and B6.Thy-1.1 mice. Con A blasts for target cells were prepared from spleen cells after removal of erythrocytes with Tris-ammonium chloride by culturing for 3 d 1.0×10^7 cells in 20 ml of RPMI 1640 medium containing 5% fetal calf serum, 2.5 μg/ml Con A, and 10% supernatant derived from secondary MLC (17). Con A blasts were incubated with α-methyl-D-mannoside before use. The assay was performed in 96-well round-bottomed microtiter plates (Costar, Cambridge, MA). After the addition of effector cells to plates containing 1.0×10^4 target cells/well, the plates were centrifuged for 3 min at 800 rpm to initiate cell contact and incubated at 37°C for 4 h. Percent specific lysis was calculated as: $100 \times$ [(cpm released with effectors − cpm released alone)/(cpm released by detergent − cpm released alone)].

Results

Effect of T Cell Subset Depletion on Skin Graft Rejection. Since previous results had indicated that the treatment of graft-immune spleen cells with anti-Lyt-2 antibody plus C′ had no effect on their ability to transfer skin graft rejection across a totally allogeneic barrier (4), we attempted to confirm these results using minor histocompatibility antigen-different skin grafts. B6.Thy-1.2 ATXBM mice were grafted with BALB.B skin and inoculated with BALB.B immune B6.Thy-1.1 spleen cell populations. By using this pair of Thy-1 congeneics, the T cell contribution of the ATXBM host could be assessed relative to the activity of the adoptively transferred T cells. The experiment shown in Table I used ATXBM hosts that had been irradiated and bone marrow reconstituted 6 wk before skin

TABLE I

*Both Lyt-1- and Lyt-2-depleted Adoptively Transferred Immune T Cells Can Induce Graft Rejection in ATXBM Mice Long After Reconstitution**

Treatment of transferred cells	Number of recipients	Graft survival (days)‡
C′ only	4	12.0 ± 0.8
Anti-Lyt-1.2 pluc C′	5	18.5 ± 2.5
Anti-Lyt-2.2 plus C′	4	17.3 ± 2.8
No cells transferred	3	>19, >26, >26§

* 6 wk after reconstitution of thymectomized, irradiated B6.Thy-1.2 mice with T cell-depleted syngeneic bone marrow, the mice were grafted with BALB.B skin and injected intravenously on the same day with 5×10^6 immune B6.Thy-1.1 spleen cells that had been subjected to the indicated treatments.

‡ Mean survival time ± SD of BALB.B skin grafts.

§ Skin graft intact on the indicated day of sacrifice.

grafting. ATXBM hosts that did not receive an inoculum of immune cells retained their grafts throughout the period of observation. Recipients of C′-treated immune cells rejected their grafts in 12 d. Prior depletion of the transferred immune spleen cells with anti-Lyt-1.2 or anti-Lyt-2.2 plus C′ allowed a slight prolongation of graft survival but both populations were able to induce rejection in all recipients.

Host T Cells Can Contribute to the Graft-specific Response in ATXBM Mice. Although untreated ATXBM graft recipients are clearly unable to reject skin grafts on their own, it was possible that the transfer of immune spleen cell populations could trigger the expansion of host T cell precursors. Thus, at the time of rejection or shortly thereafter, the spleens of grafted mice were assayed for CTL activity. On days 19 and 21 after grafting, MLC were established from the spleens of graft recipients using irradiated BALB.B spleen cells as stimulators in the presence of 10% mouse spleen Con A supernatant. After 5 d, the cultures were assayed for cytotoxicity on BALB.B and B6 target cells and the CTL effector cells were typed using Thy-1.1- and Thy-1.2-specific antibodies plus C′ (Fig. 1). In animals that received C′-treated cells or Lyt-1.2-depleted populations, the CTL response to graft-specific minor histocompatibility antigens was predominantly donor derived, i.e., the CTL activity was sensitive to anti-Thy-1.1 plus C′ treatment (Fig. 1 *A* and *B*). In these and all other cases, cytotoxicity was

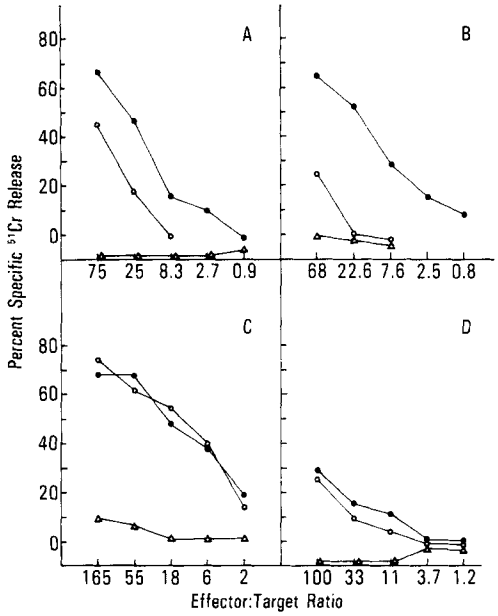

FIGURE 1. Spleen cells from ATXBM B6.Thy-1.2 mice bone marrow reconstituted 6 wk before skin grafting and adoptive transfer of immune B6.Thy-1.1 spleen cells were stimulated in culture for 5 d with irradiated BALB.B spleen cells. On the day of assay, the effector cells were treated with C′ alone (●), anti-Thy-1.1 plus C′ (○), or anti-Thy-1.1 plus anti-Thy-1.2 plus C′ (△) and assayed on BALB.B or B6 Con A blasts. No significant cytotoxicity was observed on B6 target cells. Treatments of BALB.B-immune B6.Thy-1.1 spleen cells adoptively transferred into graft recipients were as follows: (*A*) C′ only, (*B*) anti-Lyt-1 plus C′, (*C* and *D*) anti-Lyt-2 plus C′.

specific since B6 target cells were never lysed to a significant degree (data not shown). Although a percentage of the response in some of the animals that received C′-treated or Lyt-1.2-depleted cells was clearly of host origin, the most striking example of host-derived CTL activity occurred in animals receiving Lyt-2.2-depleted immune spleen cells (Fig. 1 *C* and *D*). In two of the animals that received Lyt-2-depleted cells, virtually the entire CTL response was due to host T cells (Fig. 1 *C* and *D*). In a third animal, ~50% of the CTL response was host derived while a fourth exhibited no BALB.B-specific CTL. Since ATXBM hosts not receiving immune cells did not reject their skin grafts and showed no CTL activity, these results indicate that when the graft-immune donor cells are treated with anti-Lyt-2 plus C′, the remaining cells are able to provide sufficient help to trigger host CTL precursors to, at the least, become primed to graft-specific antigens. It should be noted that all of these animals rejected their skin grafts whereas animals that did not receive sensitized cells showed no CTL activity and did not reject their grafts (Table I).

Graft Rejection in ATXBM Mice Early After Bone Marrow Reconstitution. Since host-derived T cell responses could be detected in skin-grafted ATXBM mice that had been irradiated and bone marrow reconstituted 6 wk previously, we attempted to circumvent this endogenous response by grafting mice only 1–2 wk after they received a smaller number (2×10^6) of Thy-1$^-$ bone marrow cells. Mice receiving C′-treated or anti-Lyt-1.2 plus C′-treated immune B6.Thy-1.1 cells on the day of skin grafting normally rejected grafts (range, 13–17 d) (Table II). Similarly, in the recipients of anti-Lyt-2.2 plus C′-treated cells, grafts were rejected, although the rejection time was prolonged in some cases (range, 13–21 d). Rejection was not observed if anti-Thy-1 plus C′-treated immune cells were injected, or if animals received no immune cells. Thus, when ATXBM hosts were used only 1 wk after bone marrow reconstitution at a time when host-derived T cell activity should be low, graft rejection proceeded normally in animals given either Lyt-1- or Lyt-2-depleted immune cells.

Graft-specific Cytotoxicity Is Donor Cell-derived in ATXBM Mice Used Early After Reconstitution. Functional testing of spleen cells from animals that had been

TABLE II

Skin Graft Rejection in ATXBM Mice Soon After Bone Marrow Reconstitution *

Treatment of transferred cells	Number of recipients	Graft survival (days)‡
C′ only	4	15.0 ± 0.8
Anti-Lyt-1.2 plus C′	5	14.4 ± 0.6
Anti-Lyt-2.2 plus C′	9	17.1 ± 2.7
Anti-Thy-1 plus C′	5	>19, >19, >25, >44, >44§
No cells transferred	5	>19, >21, >21, >25, >35§

* 1 wk after reconstitution of thymectomized, irradiated B6.Thy-1.2 mice with 2×10^6 T cell-depleted syngeneic bone marrow, the mice were grafted with BALB.B skin and injected intravenously on the same day with 4×10^6 immune B6.Thy-1.1 spleen cells that had been subjected to the indicated treatments.

‡ Mean survival time ± SD of BALB.B skin grafts.

§ Graft intact on indicated day of sacrifice.

grafted 1 wk after bone marrow reconstitution revealed that any CTL activity against BALB minor histocompatibility antigens was wholly donor T cell derived (Fig. 2, Table III). MLC were set up with irradiated BALB.B stimulators and medium containing 10% mouse Con A supernatant between days 19 and 26 after skin grafting. Animals that had received C′-treated (Fig. 2*A*) or Lyt-1.2-depleted cells (Fig. 2*B*) showed a high specific cytotoxicity for BALB.B targets that was completely sensitive to anti-Thy-1.1 plus C′. Unexpectedly, some animals that had received an inoculum treated with anti-Lyt-2 plus C′ also exhibited donor-derived CTL activity. In the recipients of anti-Lyt-2-treated cells, MLC cells derived from four of six animals tested gave significant graft-specific cytotoxicity while cells from two animals showed no CTL activity against BALB.B targets (Table III). All of these animals rejected their grafts and no correlation was seen between the level of cytotoxicity in MLC and the rejection time of BALB.B grafts. This point is further exemplified by the fact that some animals receiving immune cells treated with anti-Thy-1 plus C′ were also able to generate cytotoxic activity against BALB.B targets while retaining their skin grafts (Fig. 2*C*, *D*, Table II).

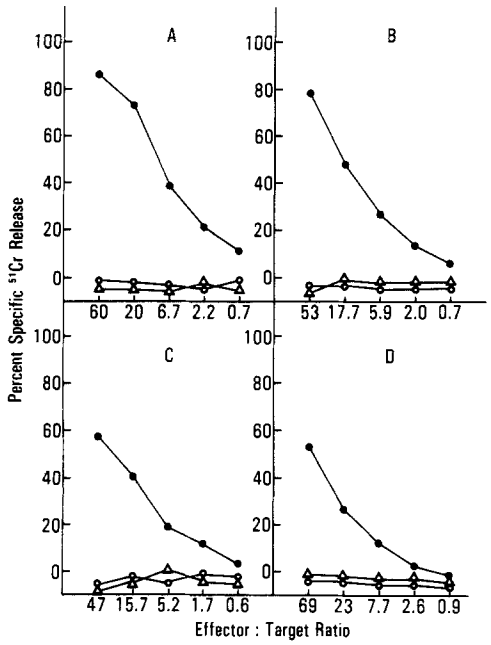

FIGURE 2. Spleen cells from ATXBM B6.Thy-1.2 mice bone marrow reconstituted 1 wk before skin grafting and adoptive transfer of immune B6.Thy-1.1 spleen cells were stimulated in culture for 5 d with irradiated BALB.B spleen cells. On the day of assay, the effector cells were treated with C′ alone (●), anti-Thy-1.1 plus C′ (○), or anti-Thy-1.1 plus anti-Thy-1.2 plus C′ (△) and assayed on BALB.B or B6 Con A blasts. No significant cytotoxicity was observed on B6 target cells. Treatments of BALB.B-immune B6.Thy-1.1 spleen cells adoptively transferred into graft recipients were as follows: (*A*) C′ only, (*B*) anti-Lyt-1 plus C′, (*C*) anti-Lyt-2 plus C′, (*D*) anti-Thy-1 plus C′.

TABLE III

*Relative Contribution of Adoptively Transferred Immune (Thy-1.1) and Host T Cells (Thy-1.2) to Graft-specific Cytotoxicity**

Treatment of transferred cells	Animal	Percentage of cytotoxicity due to donor T cells‡	Lytic units/ 10^6 cells	Graft rejection
Experiment 1				
C′	1	100	0.9	+
	2	66	9.1	+
	3	100	18.6	+
Anti-Lyt-1.2+C′	1	100	14.4	+
	2	66	0.8	+
	3	90	4.5	+
	4	100	2.7	+
Anti-Lyt-2.2+C′	1	<1	23.4	+
	2	50	0.9	+
	3	5	0.8	+
	4	—	<0.1	+
No cells transferred	1,2,3	—	<0.1	NR§
Experiment 2				
C′	1	100	29.5	+
	2	100	14.1	+
	3	100	9.8	+
Anti-Lyt-1.2+C′	1	100	47.9	+
	2	100	18.2	+
	3	100	2.1	+
	4	100	1.6	+
Anti-Lyt-2.2+C′	1	100	12.9	+
	2	100	11.5	+
	3	100	1.7	+
	4	100	1.5	+
	5	—	<0.1	+
	6	—	<0.1	+
No cells transferred	1,2,3	—	<0.1	NR

* ATXBM B6.Thy-1.2 mice were used as recipients of BALB.B skin grafts and immune B6.Thy-1.1 spleen cells 6 wk after irradiation and bone marrow reconstitution (experiment 1; see Table I) or 1 wk after irradiation and bone marrow reconstitution (experiment 2; see Table II).

‡ The percentage cytotoxicity due to donor or host CTL was calculated from comparisons of complete titrations of effector cells treated with C′ alone, anti-Thy-1.1 plus C′, or anti-Thy-1.1 plus anti-Thy-1.2 plus C′. BALB.B and B6 3 d Con A blasts were used as targets; range of spontaneous release was 17–31%. No cytotoxicity against B6 targets was observed in any of the assays. One lytic unit is defined as the number of effector cells required to achieve 30% specific lysis of target cells.

§ No graft rejection. The BALB.B skin graft was in good condition at the time the animal was sacrificed for MLC.

Discussion

The studies reported here were undertaken partly to assess the validity of using ATXBM hosts as "T cell free" models in studies of skin graft rejection. This was prompted by a number of recent studies (4, 6) that used ATXBM mice and suggested that Lyt-2^+ cells had no role in skin graft rejection, and by the knowledge that ATXBM mice are not truly T cell deficient (11). A summary of our results is shown in Table III. In direct contrast to the recent work of Loveland et al. (1, 4–6), Lyt-1 depletion of transferred graft-immune cells did not remove the ability of these cells to reject grafts (experiments 1 and 2). However, a major difference between our work and that of Loveland et al. is in the reactivity of the Lyt-1-specific antibody used. In their studies, Loveland et al. used CBA mice as recipients in which 100% of T cells were removed by treatment with anti-Lyt-1.1 plus C′ (in fact, their antibody depleted a greater number of cells than did treatment with anti-Thy-1 plus C′), while the anti-Lyt-1.2 antibody we used reacted with ~50% of B6 T cells. Indeed, anti-Thy-1 plus C′ treatment of transferred cells did prevent graft rejection. Thus, Loveland et al. were unable to functionally fractionate T cells by depletion of Lyt-1^+ cells, since this would remove all T cells, including CTL. In our experiment 1, in which ATXBM mice reconstituted 6 wk previously were used as graft recipients, depletion of Lyt-2^+ cells from the inoculum also did not affect graft rejection. This result is identical to that of Loveland et al (4), where a similar protocol was used. However, we examined the spleens of these mice shortly after graft rejection for graft-specific cytotoxicity and found that host-derived CTL were contributing significantly to the response. In mice receiving C′- or Lyt-1 plus C′-treated cells, the CTL response was predominantly donor T cell derived and no graft-specific cytotoxicity developed in animals that did not receive immune cells. The important implication of these findings is that Lyt-1^+ cells and/or accessory cells in the immune Lyt-2-depleted, adoptively transferred cells are able to trigger naive resident CTL precursors to react to graft-specific antigens. In a study using ATXBM rats that had been given an inoculum depleted of MRC-OX8^+ cells (antibody MRC-OX8 recognizes CTL and most natural killer cells), a large number of MRC-OX8^+ cells were later found in rejecting allografts (18). Although their origin was not determined, these cells were found only in rejecting allografts and not in syngeneic grafts. Thus, although the role of host-derived CTL in the rejection process is as yet unknown, we conclude that the involvement of Lyt-2^+ cells in skin graft rejection cannot be ruled out.

In an attempt to circumvent the host-derived T cell response, we used as recipients ATXBM mice that had been irradiated and bone marrow reconstituted only 1 wk previously (Table II and experiment 2, Table III). As in experiment 1, Table III, all animals that received immune cells treated with C′ alone, anti-Lyt-1, or anti-Lyt-2 plus C′, rejected their skin grafts. Animals receiving an inoculum of cells treated with anti-Thy-1 plus C′ or receiving no inoculum retained their grafts until the time of sacrifice. When the spleens of these mice were assayed for graft-specific cytotoxicity, we found the host-derived response to be undetectable. However, spleens from animals from each of the groups (except those not receiving immune cells) contained CTL specific for BALB minor histocompatibility antigens. Two important points are evident from these

results: there appears to be no strict correlation between in vitro generated cytotoxicity and graft rejection and it is difficult to totally deplete spleen cells of a particular subpopulation of T cells by conventional treatment with antibody and C′. It is possible that a small percentage of cells that are resistant to anti-Lyt-2 plus C′ or anti-Thy-1 plus C′ proliferate in vivo in response to the continual antigenic stimulation provided by the BALB.B skin graft.

Since we were unable to totally deplete adoptively transferred cells of T cell subsets and since an endogenous graft-specific response in ATXBM mice was evident in some cases, we are unable to answer the question as to which functional T cell type(s) is ultimately responsible for skin graft rejection. Recent results have shown that Lyt-1^+2^+ cells were essential for rejection of pancreatic islet allografts (19) and in local immunity against a tumor cell allograft (20). Additionally, when rat renal allografts were examined, the predominant cell type found was of the T suppressor/killer cell phenotype (21). Although it may be difficult to envision the destruction of a skin graft solely by the one on one cytolytic activity of CTL, it should be pointed out that CTL secrete lymphokines upon recognition of antigen that can activate accessory cells: All CTL clones examined so far secrete immune interferon on contact with antigen (22–24). In addition to its anti-viral action, immune interferon has macrophage-activation factor (MAF) activity (24, 25). This has been most clearly demonstrated by the use of recombinant mouse gamma interferon (25, 26). Thus, at the site of the graft bed, CTL will release MAF and recruit the activity of macrophages. It has also been recently reported that some CTL release interleukin 2 (27), though whether this lymphokine has a role at the graft site is not known. Therefore, through soluble mediators plus their own lytic activity, CTL could be efficient initiators of graft rejection. We are currently designing experiments in which a more complete removal of T cell subsets will allow us to determine if this is the case.

Summary

We have investigated which T cell subclass defined by cytolysis with monoclonal anti-Lyt-1.2 and anti-Lyt-2.2 antibodies is required to adoptively transfer the ability to reject skin grafts. B6.Thy-1.1 spleen cells immune to graft antigens were fractionated with antibody plus C′ and transferred to adult thymectomized, irradiated, bone marrow-reconstituted (ATXBM) B6.Thy-1.2 hosts that were simultaneously grafted with BALB.B skin. We found that when the ATXBM hosts were used 6 wk after irradiation and marrow reconstitution, both Lyt-1-depleted and Lyt-2-depleted immune spleen cells could transfer the ability to promptly reject skin grafts. However, such ATXBM recipients of Lyt-2-depleted cells that had rejected skin grafts were found to contain graft-specific CTL that were largely of host (B6.Thy-1.2) origin. When ATXBM hosts were used for the experiment 1 wk after irradiation and marrow reconstitution, no host-derived graft-specific CTL could be detected. However, graft rejection occurred in recipients of anti-Lyt-1- or anti-Lyt-2 plus C′-treated immune cells and specific CTL were generated from spleen cells of both groups. Thus, in the absence of a host-derived response, adoptively transferred immune Lyt-2^+ cells, either resistant to, or that escaped from, antibody plus C′ treatment, are able to expand

in response to the antigenic stimulus provided by the graft. A more complete elimination of specific T cell subclasses is therefore needed to assess the relative contribution of a particular subset to the graft rejection process.

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