ALLOSUPPRESSOR- AND ALLOHELPER-T CELLS IN ACUTE AND CHRONIC GRAFT-VS.-HOST (GVH) DISEASE

III. Different Lyt Subsets of Donor T Cells Induce Different Pathological Syndromes*

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The graft-versus-host reaction (GVHR)[1] is initiated by immune responses of donor T cells towards allogeneic histocompatibility antigens of the recipient. One of the possible outcomes of a systemic GVHR is acute graft-versus-host disease (GVHD), which is termed lethal GVHD (LGVHD) when fatal. Acute GVHD is characterized by a brief initial lymphoid stimulation during the first week of the GVHR (1–3), which rapidly thereafter turns into severe hypoplasia of the entire lympho-hemopoietic tissue (1–5). The clinical symptoms of acute GVHD include weight loss, aplastic anemia (1–4), hypogammaglobulinemia (1, 4), and sepsis (4). A different form of the GVHR is referred to as chronic GVHD. It is characterized by long-term stimulatory symptoms of the lymphoid tissue (1–4, 6), the formation of autoantibodies characteristic of systemic lupus erythematosus (SLE), and the development of various pathological lesions including immune-complex glomerulonephritis (ICGN) (3, 6–9). Both forms of GVHR are known to be induced by donor T cells (4, 6, 9–11), but little is known about the T cell subpopulations involved.

The dissection of the cellular mechanisms that are responsible for the hyperplastic and hypoplastic pathological GVH symptoms requires GVH-model systems that consistently induce either stimulatory or suppressive pathological symptoms. Several different GVH systems approaching this goal have been elaborated in our laboratory. In each system, parental strain T cells were injected into nonirradiated adult F_1 hybrid mice differing at H-2. The results of these studies indicated that those inocula of donor cells that preferentially reacted by the alloreactive T helper (T^H) cells caused the stimulatory symptoms of chronic

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[1] *Abbreviations used in this paper:* B10, C57BL/10ScSn; BDF_1, (B10 × DBA/2)F_1; C, rabbit serum complement; dsDNA, double-stranded DNA; GVHD, graft-vs.-host disease; GVH F_1, F_1 mice undergoing GVH reaction; GVHR, GVH reaction; ICGN, immune-complex glomerulonephritis; LGVHD, lethal GVHD; N F_1, noninjected F_1; NMS, normal mouse serum; PFC, plaque-forming cell(s); SLE, systemic lupus erythematosus; SRBC, sheep erythrocyte(s); T^H, T-helper; T^K, T-killer, T^S, T-suppressor.

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GVHD, whereas donor cell inocula that appeared to preferentially react by their alloreactive T suppressor (T^S) cells caused the suppressive pathological symptoms of acute GVHD (2–4, 7, 8, 10). Moreover, when unselected donor T cells were injected into F_1 recipients, which differed solely at class II H-2 antigens (I-A/I-E), exclusively stimulatory GVHD was found; in contrast, a difference in the F_1 recipients at both class I (H-2 K/D) and class II antigens was needed for the induction of acute GVHD (3, 8). Neither clear-cut stimulatory nor suppressive GVH symptoms were found in those F_1 recipients that differed from the donor solely at class I H-2 antigens (3).

From studies on the allogenetic effect in vitro it is known that class II (I-A/I-E) antigenic differences activate Lyt-1^+2^- T^H cells, which then provide maximal allohelp (12, 13). By contrast, the activation of alloreactive T^S cells requires the presence of Lyt-1^+2^+ cells as well as incompatibility, on the allogeneic B cells, at class I H-2 antigens (12, 13). Moreover, it seems from these studies in vitro that in order to become fully activated, the class I-reactive Lyt-1^+2^+ T^S cells must be induced by class II-reactive Lyt-1^+2^- cells (13, 14). These findings suggest that the class II (I-A/I-E) incompatibilities in the F_1 recipients previously studied (3, 8) selectively activated the alloreactive T^H cells out of the pool of unselected donor T cells; the activated T^H cells might then have caused the formation of SLE-like autoantibodies and the development of other stimulatory GVH symptoms. In contrast, an incompatibility in the F_1 recipient at both class II (I-A/I-E) and class I (K/D) antigens might have activated both the class II-reactive T^H or inducer cells and the class I-reactive T^S effector cells (3). It should be noted here that alloreactive donor T^S cells, and not cytotoxic T cells, appear to be important effector cells in acute GVHD. These T^S cells seem to be the effector cells, because they suppress the physiologic proliferation of lympho-hemopoietic cells and thus cause the pancytopenia of acute GVHD (4).

The intra-H-2 requirements for the induction of either stimulatory (chronic) GVHD or suppressive (acute) GVHD (3, 8) parallel those for the induction of positive and negative allogeneic effects in vitro (12, 13). The missing link between the studies performed in vitro and the GVH experiments in vivo concerns the Lyt subsets of donor T cells that are required for the induction of either stimulatory or suppressive GVHD. We therefore injected different Lyt subsets of B10 donor T cells into (B10 × DBA/2)F_1 (BDF_1) mice. The results indicate that Lyt-1^+2^- T cells are able to induce SLE-like GVHD, but are incapable of inducing acute GVHD and LGVHD. The induction of acute GVHD and LGVHD on the other hand required unseparated donor T cells, which comprise both Lyt-1^+2^- and Lyt-1^+2^+ cells.

Materials and Methods

Mice. B10 mice (H-$2^{b/b}$, Lyt-1.2, Lyt-2.2, Thy-1.2) and BDF_1 hybrids (H-$2^{b/d}$) were purchased from Olac 1976 Ltd. (Bicester, Oxon, U.K.). Female mice, 8–10-wk old, were used.

Preparation of Donor Cells. For induction of GVHR, single-cell suspensions of donor spleen and lymph node cells were prepared, as described (7).

Monoclonal Antibodies. Monoclonal murine anti-Lyt-1.2 (IgG2b, no. NEI-017) and anti-Lyt-2.2 (IgM, No. NEI-006) antibodies were obtained from New England Nuclear (Boston, MA). Monoclonal murine anti-Thy-1.2 (IgM, clone F7D5) was purchased from Olac 1976

Ltd. (Bicester, Oxon, U.K.).

Pretreatment of Donor Cells with Monoclonal Antibodies and Complement (C). Donor cells consisted either of spleen cells or of a mixture of spleen cells (2 parts) and lymph node cells (1 part). For treatment with antiserum, cell suspensions were prepared in Hepes-buffered RPMI 1640 that contained 5% fetal calf serum. Suspensions of 3×10^7 live cells/ml were incubated for 45 min on ice with either anti-Thy-1.2 serum, diluted 1 in 1,000, or anti-Lyt-1.2 or anti-Lyt-2.2 serum, each diluted 1 in 3,000. As a control, cells were treated with normal mouse serum (NMS) diluted 1 in 3,000. After incubation, the cells were spun down and resuspended with 6 ml rabbit C, which had been selected for low cytotoxicity and was diluted 1 in 12 in Hepes-buffered RPMI 1640 (not containing fetal calf serum). The suspensions were incubated for 45 min at 37°C, and thereafter the cells were washed three times. In some experiments the donor cells were treated twice with antisera and C, according to this procedure. Hereafter, cells treated with anti-Lyt-1.2 and C, anti-Lyt-2.2 and C, anti-Thy-1.2 and C, or NMS and C will be referred to as anti-Lyt-1-, anti-Lyt-2-, anti-Thy-1-, and NMS-treated cells, respectively. Unless mentioned otherwise, these cells had been treated once.

Nylon-wood Passage of Pretreated Donor Cells. In one experimental approach, B10 spleen cells, which had been pretreated twice with either NMS or anti-Lyt antibodies, were passed through nylon-wool columns (13) in order to reduce the fraction of non-T cells.

Induction of GVHR. All donor cells were injected intravenously. In the first experiment (Fig. 1 and Table I) nonirradiated mice were used as recipients. In the second experiment (Fig. 3 and Table II) the prospective BDF_1 recipients were first irradiated with 750 rad by using 662-KeV gamma rays, emitted from a ^{137}Cs source (gammator, model 381, Isomedic) at a dose of 375 rad/min. Within the next 3 h, these mice were repopulated with 3×10^7 live untreated spleen cells obtained from syngeneic (BDF_1) donors; 1 d later, the GVHR was induced by injection of B10 T cells. The donor cell inocula are specific in the results section.

Primary Anti-Sheep Erythrocyte (SRBC) Response. The primary plaque-forming cell (PFC) response to SRBC in vitro was performed as described elsewhere (13).

Clinical Signs of GVHR. Twice a week, the F_1 mice undergoing GVHR (GVH F_1) were inspected for symptoms of acute GVHD, such as weakness, diarrhea, and ruffled fur; weight loss was determined by weighing the mice weekly. The number of dead mice was recorded. Anemia was determined by two-weekly measurements of hematocrit. At weekly intervals, the GVH F_1 mice were tested for elevated proteinuria ($\geq$300 mg protein per 100 ml urine) by means of Albustix test sticks (no. 2872, Ames, Div. of Miles). Elevated proteinuria in GVH F_1 mice is a reliable indicator of ICGN (9).

Detection of Autoantibodies. The presence of autoantibodies in the serum was determined at two-weekly intervals, starting at week 2 and lasting until week 16 after the initiation of the GVHR. Autoantibodies to erythrocytes were detected by the direct Coombs' test using anti-mouse IgG serum, as described (7). Autoantibodies to thymocytes were detected by a C-dependent cytotoxicity test, as described (7); using ^{51}Cl-labeled thymocytes of normal BDF1 mice as target cells. Mice were scored positive for autoantibodies against thymocytes when a 1 in 5 dilution of their serum lysed 40% or more of the target cells. IgG antibodies against nuclear antigens were detected by an indirect immunofluorescence technique using cryostat sections of mouse liver as antigenic substrate, as described (7). IgG autoantibodies against double-stranded DNA (dsDNA) were determined by an indirect immunofluorescence technique using the extranuclear dsDNA of *Crithidia luciliae* as antigenic substrate, as described (7). For the determination of both antinuclear and anti-dsDNA antibodies, the initial dilution of mouse serum was 1 in 10; the highest serum dilution at which specific immunofluorescence was seen was called the titer.

Results

Lyt Subsets of Donor T Cells Involved in the Induction of LGVHD in Nonirradiated F_1 Mice. In order to determine which donor T-cell subset(s) is (are) responsible for the induction of LGVHD, groups of nonirradiated BDF_1 mice were injected

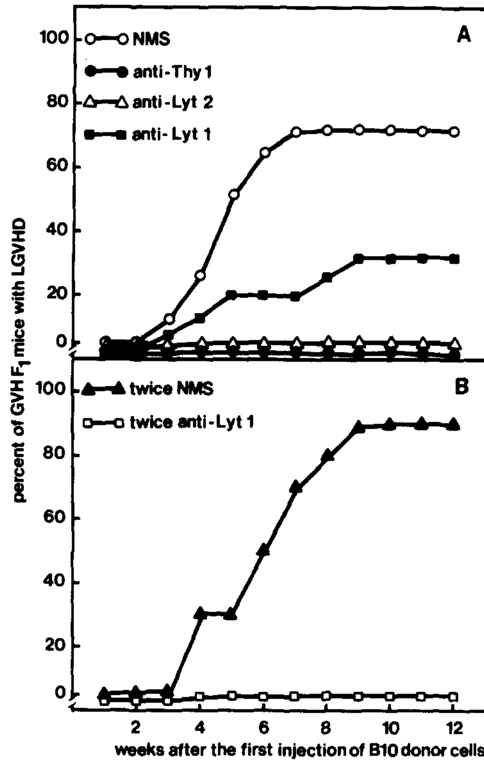

FIGURE 1. Induction of LGVHD as a function of the Lyt subsets of parental T cells administered. Groups of nonirradiated BDF_1 mice were injected on both day 0 and day 7 with a mixture of 5×10^7 viable B10 donor cells; the original mixture was composed of one-third lymph node cells and two-thirds spleen cells. The B10 donor cells had been pretreated either once (*A*) or twice (*B*), as indicated. Each of the groups of BDF_1 mice, which had received the donor cells pretreated once, consisted of 15 mice; the groups of BDF_1 mice, which had received the donor cells pretreated twice, consisted of 10 mice each.

with various inocula, each of which contained 10^8 viable B10 cells. As expected, the vast majority (73%) of the F_1 recipients of B10 cells that had been treated once with NMS succumbed to a typical acute GVHD (Fig. 1*A*); their LGVHD was always preceded by a 20–30% reduction of the hematocrit values (hematocrit values of N F_1 mice ranging from 43 to 48). None of the F_1 recipients of anti-Thy-1-treated B10 cells developed acute GVHD, not to mention LGVHD (Fig. 1*A*); consistent with this observation, the hematocrit values of these F_1 recipients were normal (ranging from 42 to 48). Treatment of the B10 donor cells with anti-Lyt-2 completely abolished their capacity to induce acute GVHD and LGVHD; none of the GVH F_1 mice of this group had reduced hematocrit values. After a single treatment of the B10 donor cells with anti-Lyt-1, one-third of the F_1 recipients still developed acute GVHD (hematocrit values between 30 and 35) and eventually died from acute GVHD (Fig. 1*A*). After the donor cells had been treated twice with anti-Lyt-1, however, the inoculum of 10^8 viable B10 cells had lost its capacity to induce LGVHD (Fig. 1*B*) and even acute GVHD (hematocrit values ranging from 43 to 48). Donor cells, which had been treated twice with

TABLE I

Development of SLE-like GVHD in Nonirradiated BDF_1 Mice as a Function of the Lyt Subset of Parental T Cells Administered

Group	Pretreatment of B10 donor cells‡	Nonirradiated BDF_1 recipients:* Cumulative percentage of surviving mice (week 16) with autoantibodies against					Percentage of mice with ICGN¶
		Number of mice tested§	Thymocytes	Erythrocytes	Nuclear antigens (titer)‖	dsDNA (titer)‖	
1	NMS	4	0	0	50 (1/40–1/160)	0 (–)	0
2	Anti-Thy-1	15	0	0	20 (1/20–1/160)	0 (–)	0
3	Anti-Lyt-1	10	10	0	20 (1/40–1/160)	0 (–)	0
4	Twice anti-Lyt-1	10	0	0	10 (1/80)	0 (–)	0
5	Anti-Lyt-2	15	100	93	100 (1/160–1/2,560)	40 (1/20–1/160)	33

* All mice received a total of 10^8 viable donor cells; the cells were administered in two equal portions on day 0 and day 7. The original donor-cell inocula were composed of 2 parts of spleen cells and 1 part of lymph node cells.

‡ Unless mentioned otherwise, the donor cell inocula were pretreated once. After treatment, all inocula were adjusted to the number of viable cells.

§ Represents the number of mice that did not die from acute GVHD (see Fig. 1). Group *1*, *2*, *3*, and *5* initially consisted of 15 recipient mice; group *4* initially consisted of 10 recipient mice.

‖ Range of maximal titers in positive mice.

¶ Mice showing elevated proteinuria for at least 3 subsequent weeks as well as distinct granular deposits of IgG along the glomerular basement membrane when examined by immunofluorescence technique, as described elsewhere (9).

NMS, had retained their capacity to induce LGVHD (Fig. 1*B*). These findings indicate that the donor T cells involved in the induction of acute GVHD must be Lyt-2^+ as well as Lyt-1^+.

The Donor T Cells That Cause SLE-Like GVHD in Nonirradiated F_1 Recipients Carry the Lyt-1^+2^- Phenotype. The above-described nonirradiated F_1 recipients, which had been studied for acute GVHD and LGVHD, were also studied for the development of SLE-like GVHD (Table I). In addition, we studied control groups, each of which consisted of eight syngeneically injected BDF_1 or B10 recipient mice. The syngeneic cells injected were either NMS-, anti-Lyt-1-, or anti-Lyt-2-treated. None of these control mice produced antibodies to thymocytes, erythrocytes, or dsDNA, let alone developed ICGN. Only one or two animals out of each of these groups produced autoantibodies against nuclear antigens. The maximal titer of these antibodies ranged from 1 in 40, to 1 in 160 (data not shown).

All groups of F_1 mice injected with antiserum-treated B10 donor cells initially consisted of 10 or 15 mice but, as already described in Fig. 1, many of the F_1 recipients in groups *1* and *3* of Table I had died from acute GVHD before the end of the experiment (week 16 after the first administration of B10 cells). However, none of these mice showed detectable amounts of lupus-like autoantibodies in their sera or had developed elevated proteinuria as a sign of ICGN (data not shown).

Group *5* in Table I shows that treatment with anti-Lyt-2 rendered the B10 cells capable of inducing an SLE-like autoimmune disease in the BDF_1 recipients. Already 2 wk after the first injection of donor cells, 53% of the F_1 mice had autoantibodies to thymocytes, nuclear antigens, and erythrocytes. At 4 wk after the induction of the GVHR, 100% of the F_1 recipients had autoantibodies to thymocytes and nuclear antigens in their serum, and 93% were Coombs'-positive. All F_1 recipients in this group remained positive for autoantibodies to thymocytes

and nuclear antigens until week 16. Moreover, 40% of the F_1 recipients had autoantibodies to dsDNA in their serum at one or more times tested; and severe ICGN, as indicated by elevated proteinuria and immunofluorescence studies, was observed in 33% of the F_1 recipients.

In marked contrast, none of the other GVH F_1 mice shown in Table I (groups *1* to *4*) had comparable symptoms of SLE-like GVHD. None of them showed elevated proteinuria or immunohistological evidence of ICGN, and none of them had autoantibodies against erythrocytes and dsDNA. The titers of antinuclear antibodies, if present at all, did not exceed the titers found in syngeneically injected control mice. Although the percentage of GVH F_1 mice with antinuclear antibodies was increased in the F_1 recipients of NMS-treated B10 donor cells (group *1*), this percentage (50%) represents only two long-term survivors out of the initial group of 15 GVH F_1 mice. As expected, treatment of the B10 donor cells with anti-Lyt-1 did not render them capable of inducing SLE-like GVHD (groups *3* and *4*).

Subpopulations of Donor T Cells Involved in the Induction of LGVHD in Irradiated F_1 Recipients. Since ~70% of the peripheral T cells in the mouse express the Lyt-2 antigen (15), the total number of T cells among 10^8 viable Lyt-2-depleted B10 cells comprised only about one-third of the total number of T cells in NMS-treated cells. This total number of donor T cells after the anti-Lyt-2 treatment might therefore have been too low to induce acute GVHD and thus caused SLE-like GVHD in the nonirradiated F_1 recipients used. This explanation is unlikely though because small doses of untreated B10 donor cells (5×10^6 to 30×10^6, 2 parts spleen cells and 1 part lymph node cells) induced only a limited autoantibody formation and failed to induce SLE-like GVHD (data not shown). To further exclude this possibility comparable numbers of viable antiserum-treated B10 T cells were injected into irradiated BDF_1 recipients. In order to adjust the various donor cell inocula to comparable numbers of viable T cells, B10 spleen cells that had been treated twice with either NMS, anti-Lyt-1, or anti-Lyt-2, were passed through nylon-wool columns.

First, we determined the alloreactivity of these three suspensions of B10 T cells by means of the primary anti-SRBC response in vitro (Fig. 2). Low numbers of NMS-treated B10 T cells induced allohelp; higher numbers induced allosuppression. The anti-Lyt-2-treated B10 T cells exclusively induced allohelp, and the anti-Lyt-1-treated B10 T cells were ineffective. These results are consistent with those reported elsewhere (12, 13).

Fig. 3 shows the different capacities of the same three suspensions of B10 T cells to induce LGVHD in irradiated BDF_1 mice were used, because they irradiated F_1 recipients are much more susceptible than nonirradiated ones to the induction of LGVHD (11, 16). Whereas 90% of the F_1 recipients of the NMS-treated B10 T cells developed LGVHD, only one (10%) of the recipients of anti-Lyt-2-treated T cells died, and none of the F_1 recipients of anti-Lyt-1-treated B10 T cells died.

Subset of Donor T Cells Causing Chronic GVHD in Irradiated F_1 Mice. The same groups of irradiated BDF_1 mice that had been used for studying LGVHD were also used for testing the presence of SLE-like autoantibodies in the serum (Table II). None of the F_1 recipients that died from acute GVHD before the end of the

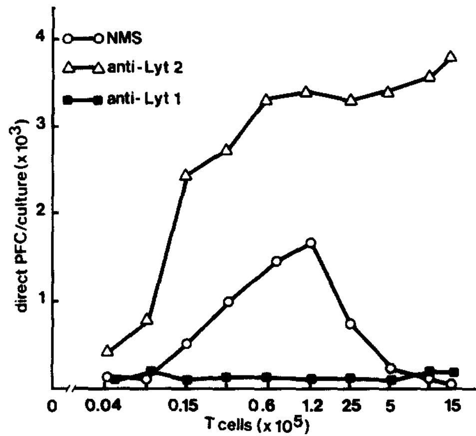

FIGURE 2. Allogeneic effects caused by Lyt subsets of B10 T cells in vitro. The effects of B10 T cells on the primary PFC responses of SRBC-stimulated BDF1 B cells were compared. Each culture contained 3×10^6 T cell-depleted BDF_1 spleen cells (B cells). As a source of T cells, graded numbers of live B10 spleen cells that had been treated twice, as indicated, were used; after the second treatment, these cells were passed through nylon-wool columns, adjusted to the same number of live cells, and added to the B cell cultures. The three T-cell suspensions tested here are the same that were used to perform the GVH experiments described in Fig. 3 and Table II.

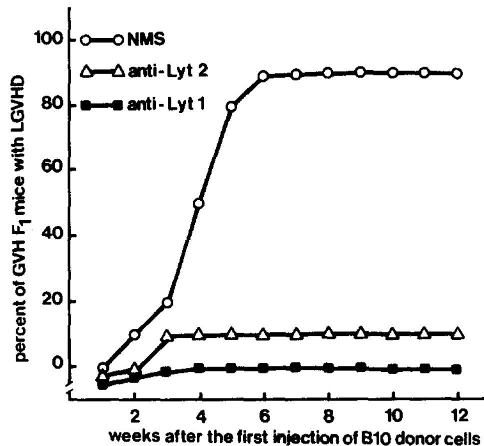

FIGURE 3. Induction of LGVHD in irradiated BDF_1 recipients as a function of the Lyt subsets of parental T cells administered. Groups of 750-rad irradiated BDF_1 mice were first repopulated with 3×10^7 syngeneic (BDF_1) spleen cells and on the following day injected with 10^7 viable B10 cells as a source of T cells. The T cells were obtained from B10 spleen cells that had been pretreated twice, as indicated; thereafter, the cells had been passed over nylon-wool. The resulting cell suspensions were adjusted to the same concentration of live cells and then injected into the recipients. Each group of recipients initially consisted of 10 F_1 mice.

observation period (week 16) had shown autoantibodies before they died. In Table II, therefore, only the results obtained from the surviving GVH F_1 mice are shown. It is evident from the Table that the vast majority of the F_1 recipients of anti-Lyt-2-treated donor cells showed a vigorous formation of SLE-like auto-

TABLE II
Formation of SLE-like Autoantibodies in Irradiated BDF$_1$ Mice as a Function of the Lyt Subsets of B10 T Cells Administered

T cells obtained from B10 donors*		Irradiated BDF$_1$ recipients:‡ Cumulative percentage of surviving F$_1$ mice with autoantibodies against				
Number of live cells administered ($\times 10^6$)	Pretreatment of cells	Number tested§	Thymocytes	Erythrocytes	Nuclear antigens (titer)‖	dsDNA (titer)‖
0	—	10	0	0	20 (1/20–1/160)	0 (–)
10	NMS	1	0	0	100 (1/160)	0 (–)
10	Anti-Lyt-1	10	0	0	20 (1/40–1/160)	0 (–)
10	Anti-Lyt-2	9	100	78	100 (1/40–1/2,560)	78 (1/40–1/80)

* Same cells as those used for the experiments described in Figs. 2 and 3.
‡ Same mice as those used for the experiment shown in Fig. 3.
§ Represents the numbers of F$_1$ recipients that had survived until the end of the experiment, i.e. week 16, after the injection of B10 cells. At the start of the experiment, each group consisted of 10 F$_1$ mice.
‖ Range of maximal serum titers of positive F$_1$ mice.

antibodies. In contrast, neither NMS-treated nor anti-Lyt-1-treated B10 T cells were capable of inducing a comparable autoantibody formation in the recipients.

Discussion

The results of the present study indicate that, depending on the Lyt phenotype of the parental T lymphocytes injected, the F$_1$ recipients developed either acute or chronic GVHD.

Stimulatory or Chronic GVHD. In previous studies, it was shown that injection into nonirradiated BDF$_1$ recipients of unseparated B10 T cells led to only limited autoantibody formation (2) and failed to induce ICGN but, instead, caused acute GVHD and LGVHD (10). Using the same parent → F$_1$ combination, now we showed that depletion of the allosuppressive Lyt-2$^+$ cells rendered the B10 donor cells capable of inducing a severe SLE-like autoimmune disease that included the development of ICGN (Table I). The mechanism underlying the formation of autoantibodies during the GHVR is thought to be a positive allogeneic effect caused by abnormal T-B-cell cooperation (3, 7, 8, 17–21). In this process, T cell help is provided by parental T cells that react against the allogeneic class-II (I-A/I-E) antigens of the F$_1$ recipient's H-2 complex (3, 8). The excess of T cell help generated by this reaction, in combination with certain self-antigens, triggers those F$_1$ B cells capable of producing autoantibodies characteristic of SLE (3, 7, 8, 17, 21). The autoantibodies in turn form immune complexes and cause ICGN (9). In the present study, we showed that the production of multiple autoantibodies and ICGN during a GVHR requires the injection of Lyt-1$^+$2$^-$ donor cells; that Lyt phenotype is commonly associated with T^H cells (15, 22, 23), including the T^H cells that react against allogeneic class-II antigens (Fig. 2; 12, 13). In contrast, the B10 cells that had been pretreated with anti-Lyt-1 were unable to induce vigorous autoantibody formation in the recipients (Table I and II). The latter finding is hardly surprising, however, because virtually all T cells express

at least small amounts of Lyt-1 (24). The low level of antinuclear antibodies observed after the injection of anti-Lyt-1-treated cells barely exceeded that found in syngeneically injected or noninjected BDF_1 mice. This low level of spontaneous autoantibody formation conforms with previous reports (25, 26) that normal mice, especially at higher ages, may have antinuclear antibodies in the serum.

The different forms of GVHD induced by different Lyt subsets of donor T cells argue against the pathogenesis of chronic GVHD postulated by Elkins (1, 27). He proposed that the symptoms of chronic GVHD are only late indirect consequences of the cytotoxic damage and the subsequent infections caused by the initial alloaggression by donor T cells. The data presented in the present paper, however, as well as other data (2–4, 7, 8) indicate that stimulatory GVH symptoms can appear already at 2 wk after the initiation of the GVHR and do not require a preceding phase of lymphohemopoietic depletion (acute GVHD); instead, these symptoms are direct consequences of the allo-stimulation induced by donor T^H cells. In fact, we observed that removing the Lyt-2^+ donor T cells, the very cells that are needed for the induction of acute GVHD, was a prerequisite for the induction of SLE-like GVHD by B10 T cells. Nevertheless, viral infections, including those acquired during GVHD, might lead to a GVH-like pathogenesis so that their symptoms are (almost) indistinguishable from those caused by alloreactive donor T cells (6, 17, 21).

Acute GVHD. Following an initial phase of stimulatory symptoms in the first week of the GVHR (1, 2), acute GVHD rapidly produces suppressive pathological symptoms, such as pancytopenia accompanied by aplastic anemia and hypogammaglobulinemia (1–5, 10). This biphasic course has led us to the concept (2) that acute GVHD in H-2-different recipients is due to a sequential activation, first of class-II-reactive donor T^H or T inducer cells and, thereafter, of class-I-reactive donor T^S cells. The activated donor T^H cells might cause the initial phase of stimulatory symptoms, and, analogous to other systems (14), induce class-II-reactive donor T^S cells. Optimally activated donor T^S cells appear to cause the suppressive pathological symptoms of GVHD (2–4, 10, 28, 29).

In the present paper, we showed the alloreactive B10 cells carrying the Lyt-1^+2^- phenotype were unable, or almost unable, to induce acute GVHD and LGVHD in either nonirradiated or irradiated H-2-different F_1 mice (Figs. 1 and 3). This finding is consistent with the results published by Korngold and Sprent (30), who showed that Lyt-1^+2^- donor T cells were unable to induce LGVHD in irradiated recipients differing from the donor at multiple non-H-2 loci. Our findings differ, however, from the studies reported by Valera et al. (16). Valera et al. studied the Lyt phenotype of the donor T cells responsible for the induction of LGVHD in irradiated recipients, which differed from the donor at all of H-2 as well as non-H-2 loci. They found that treatment of the donor cells with anti-Lyt-2 did not protect the recipient mice from LGVHD. The most likely explanation for this discrepancy with our data is that the single anti-Lyt-2 treatment of the donor cells used by Valera et al. (16) was not sufficient to remove all of the Lyt-2^+ T cells and that the few spared T cells were responsible for the observed LGVHD in the irradiated recipients. In our hands, a single treatment with anti-Lyt-2 or alloreactive T cells often proved to be ineffective in abolishing their allosuppressor capacity in vitro (data not shown). In fact, Valera et al. (16)

reported that their treatment with anti-Lyt-2 did not completely abrogate the capacity of the donor cells to generate anti-host T killer cells in vitro. Others (15, 31), by contrast, have shown that T cells properly depleted of Lyt-2^+ cells were no longer capable of generating cytotoxic T cells across an H-2 difference. The possibility that, indeed, a very low number of spared Lyt-2^+ T cells might have caused the LGVHD observed by Valera et al. (16) receives further support by previous findings made by Korngold and Sprent (11). These authors observed that parental bone marrow cells, which contained as few as 0.3% mature T cells, i.e. 3×10^4 T cells, sufficed to induce LGVHD in irradiated F_1 recipients that differed at multiple non-H-2 loci only. Moreover, very similar observations have recently been made in human recipients of HLA-different bone marrow grafts (32).

In agreement with Korngold and Sprent (30) and Valera et al. (16) we found that Lyt-1^-2^+ T cells, too, are unable to induce LGVHD. Together with the observed inability of Lyt-1^+2^- cells to induce LGVHD, the combined results lead to the conclusion that donor T cells carrying the Lyt-1^+2^+ phenotype must be involved in the pathogenesis of LGVHD. This conclusion is identical with that of Korngold and Sprent (30). It is not yet known, however, whether Lyt-1^+2^- inducer T cells are required in addition to the Lyt-1^+2^+ donor cells.

As already mentioned, we think that alloreactive donor T^S cells are the effector cells that cause the pancytopenia of acute GVHD (3, 4, 10). Our conclusion that donor Lyt-1^+2^+ cells must be involved in the pathogenesis of LGVHD is consistent with this concept, because several groups of investigators showed that the severe allosuppression at the beginning of the second phase of acute GVHD, i.e. at about day 7, is caused by Lyt-1^+2^+ donor T cells (33–35). Furthermore, recently we provided evidence that allosuppression induced in vitro also requires Lyt-1^+2^+ cells, because neither Lyt-1^-2^+ cells alone nor a mixture consisting of Lyt-1^+2^- cells and increasing numbers of Lyt-1^-2^+ cells were able to induce allosuppression across a full H-2 difference (13). These studies also showed that a class-II-reactive Lyt-1^+2^- cell causes allohelp, whereas allosuppression in vitro appears to be caused by a class-I-reactive Lyt-1^+2^+ cells. Thus, the data of the present study have closed the gap between the studies performed in vitro (13) and the GVH experiments performed in vivo. In combination with previous findings (3, 8), we conclude that stimulatory GVHD is caused by class-II-reactive Lyt-1^+2^- donor T^H cells. In contrast, the development of suppressive GVHD seems to require class-I-reactive Lyt-1^+2^+ T^S cells and, possibly also class II-reactive Lyt-1^+2^- inducer cells of the donor. However, experiments involving positively selected Lyt-1^+2^+ cells will have to be performed in order to verify this concept.

Summary

Previous work from this laboratory has led to the hypothesis that the stimulatory pathological symptoms of chronic graft-vs.-host disease (GVHD) are caused by alloreactive donor T helper (T^H) cells, whereas the suppressive pathological symptoms of acute GVHD are caused by alloreactive T suppressor (T^S) cells of the donor. In the present paper we analyzed the Lyt phenotypes of B10 donor T cells required for the induction of either acute or chronic GVHD in H-2-

different (B10 × DBA/2)F_1 recipients. First, nonirradiated F_1 mice were used as the recipients. We found that unseparated B10 T cells induced only a moderate formation of systemic lupus erythematosus (SLE)-like autoantibodies, but a high percentage of lethal GVHD (LGVHD). In contrast, Lyt-1^+2^- donor T cells were unable to induce LGVHD in these recipients; these cells were capable, however, of inducing a vigorous formation of SLE-like autoantibodies and the formation of severe immune-complex glomerulonephritis. Lyt-1^-2^+ T cells were incapable of inducing either acute or chronic GVHD.

In another experiment, the sensitivity and accuracy of the GVH system were increased by using irradiated F_1 mice as recipients and by comparing donor-cell inocula that contained similar numbers of T lymphocytes. In addition, donor-cell inocula were used that had been tested for their allohelper and allosuppressor effects on F_1 B cells in vitro. In the irradiated F_1 recipients, too, unseparated donor T cells were superior to T cell subsets in inducing LGVHD; Lyt-1^-2^+ donor cells were completely and Lyt-1^+2^- donor cells were almost incapable of doing so. In contrast, Lyt-1^+2^- T cells, but neither unseparated T cells nor Lyt-1^-2^+ T cells, were capable of inducing a vigorous formation of SLE-like autoantibodies. We conclude that the stimulatory pathological symptoms of chronic GVHD are caused by Lyt-1^+2^- allohelper T cells. In contrast, the development of the suppressive pathological symptoms of acute GVHD appears to involve alloreactive Lyt-1^+2^+ T suppressor cells.

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