# CAPACITY OF GENETICALLY DIFFERENT T LYMPHOCYTES TO INDUCE LETHAL GRAFT-VERSUS-HOST DISEASE CORRELATES WITH THEIR CAPACITY TO GENERATE SUPPRESSION BUT NOT WITH THEIR CAPACITY TO GENERATE ANTI-$F_1$ KILLER CELLS

## A Non-H-2 Locus Determines the Inability to Induce Lethal Graft-Versus-Host Disease*

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One of the possible consequences of the graft-versus-host reaction (GVHR)[1] in animals and man is runt disease or acute graft-versus-host (GVH) disease (GVHD). It is frequently fatal and produces a variety of symptoms, which include atrophy of the skin and the gastrointestinal mucosa and hypoplasia of the hemopoietic and lymphoid tissues manifested by anemia and immunodeficiency, respectively (1, 2). Donor T cells are known to be responsible for the induction of runt disease (3, 4). However, the mechanisms by which T cells induce the pathological symptoms of runt disease are poorly understood.

Another possible outcome of the GVHR is a chronic GVHD which can lead to different conditions, such as lymphadenopathy (5, 6), immune complex glomerulonephritis (6, 7), and formation of multiple autoantibodies (8–11). Previous analyses performed in nonirradiated $F_1$ mice have shown that there are two minimal, essential requirements for the induction of the symptoms of chronic GVHD: immunocompetent T lymphocytes must be present in the donor cell inoculum and they must be able to react to incompatible H-2 structures of the recipient (5–7, 9, 11).

Undefined genetic factors of the T cell-donor strain decide which of the two kinds of GVHD develop in the $F_1$ hybrid recipients (5–7, 11). This point is best exemplified by previous studies on groups of identical (C57BL/6 × DBA/2)$F_1$ or (C57BL/10 × DBA/2)$F_1$ hybrid mice as recipients of T cells from the donor strains DBA/2, C57BL/6, C57BL//10, and B10.D2. When DBA/2 T cells were injected into these $F_1$ hybrids, a syndrome ensued that resembled systemic lupus erythematosus (7, 11). However, lymphadenopathy only rarely, and runt disease never, belonged to the spectrum of abnormalities induced by the donor DBA/2 (5, 11).

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[1] *Abbreviations used in this paper:* BMC, bone marrow cells; C, complement; CML, cell-mediated lymphocytotoxicity; GVH, graft-versus-host; GVHD, GVH disease; GVHR, GVH reaction; [$^{125}$I]UdR, 5-[$^{125}$I]-iodo-2′-deoxyuridine; MLR, mixed lymphocyte reaction; $T^K$, killer T [cells].

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In contrast, when the other parental strains C57BL/6 and C57BL/10 were used as donors for groups of the same types of $F_1$ recipients, a different spectrum of GVHD developed. This involved lymphadenopathy and lymphomas (5, 6) and a limited formation of autoantibodies (11), whereas severe immune complex glomerulonephritis never developed (7). In addition, the development of severe runt disease as an unwanted side effect of GVHR was frequently observed (5, 11). As far as has been studied, a syndrome similar to that induced by C57BL/6 or C57BL/10 was evoked by the injection of spleen cells from strain B10.D2 into (C57BL/10 × DBA/2)$F_1$ recipients (5, 7, 11).

These genetic differences in the disease-inducing qualities of donor T cells from strains C57BL/10 and B10.D2 versus strain DBA/2 deserve a more detailed investigation. Therefore, we decided to study systematically the effects of GVHR, induced by either strain DBA/2 or strains on a B10-genetic background, upon groups of identical $F_1$ hybrid recipients.

## Materials and Methods

*Mice.* C57BL/10ScSn mice and (C57BL/10 × DBA/2)$F_1$ hybrids were purchased from the T.N.O. Laboratory (Zeist, The Netherlands). B10.D2nSn and DBA/1 mice were purchased from The Jackson Laboratory (Bar Harbor, Maine). DBA/2 mice were purchased from Gl. Bomholtgård Ltd. (Ry, Denmark). Female B10.BR, B10.S, B10.G, B10.M and B10.A mice were purchased from Olac Ltd. (Bicester, Oxfordshire, England). All $F_1$ hybrid mice as well as [(B10.D2 × DBA/2)$F_1$ × B10.D2] backcross mice were bred in our animal facilities, (Central Laboratory of The Netherland Blood Transfusion Service, Amsterdam, The Netherlands). Unless mentioned otherwise, female $F_1$ hybrids, 8–10 wk old, were used as GVH recipients. Female DBA/2, C57BL/10, B10.D2, B10.A, or backcross mice, 8–10 wk old, were used as donors. Female B10.BR and B10.G were first used to breed $F_1$ hybrids with male DBA/2 and, at the age of 5–6 mo, they were used as GVH donors.

*Donor Cells for Induction of GVHR.* Mice were killed and their spleens or lymph nodes (cervical, axillary, inguinal, and mesenteric) excised. The organs were minced in sterile, buffered-salt solution. Single-cell suspensions were prepared by gently passing the minced organs through a nylon sieve and, thereafter, through Pasteur pipettes filled with 1 cm of loosely packed sterile nylon wool. Bone marrow cells (BMC) were flushed with a needle and a syringe from the femoral and tibial cavities of donor mice and collected in sterile Hepes-buffered RPMI-1640.

*Enrichment of Splenic T Cells.* To enrich for splenic T cells, the method of Julius et al. (12) was used. Immunofluorescence studies showed that the cells not adhering to nylon wool usually contained 3–4% B cells, as determined by $F(ab)_2$ rabbit anti-mouse Ig serum labeled with fluorescein isothiocyanate, and 60–80% T cells, as determined by applying an appropriately absorbed rabbit anti-mouse brain serum in combination with swine anti-rabbit IgG labeled with fluorescein isothiocyanate (catalogue No. F 2190, Dakopatts, Denmark). The viability of cells was determined by trypan blue exclusion; it was never <90%.

*Depletion of T Cells.* For the depletion of T cells, spleen-cell suspensions were prepared in Hepes-buffered RPMI-1640 that contained 5% fetal calf serum. 6-ml suspensions containing 30 × $10^6$ living cells/ml were incubated for 30 min at room temperature with monoclonal anti-Thy-1.2 serum (clone F7D5; Olac Ltd.) at a dilution of 1:1,000. As a control, spleen cells from the same suspension were treated with normal mouse serum at a dilution of 1:1,000. Both suspensions were spun down and resuspended with 6 ml rabbit complement (C) selected for low toxicity and diluted 1:30 in Hepes-buffered RPMI-1640. After incubation for 30 min at 37°C, the cells were washed three times. The percentages of T cells present in the anti-Thy-1.2-treated spleen-cell suspension from strain C57BL/10 was never >1%, whereas the spleen cells treated with normal mouse serum showed identical numbers of T cells as the untreated spleen cells, i.e., 30% (range 27–35%).

*Generation of $F_1$-activated Donor T Cells in Irradiated $F_1$ Hybrids.* 50 × $10^6$ T cell-enriched donor

spleen cells were injected i.v. into groups of irradiated (C57BL/10 × DBA/2)$F_1$ hybrids, 10–14 wk old. 1–3 h before injection, the $F_1$ recipients were irradiated with 750 rad by using 662 keV gamma rays delivered by a $^{137}Cs$ source. 5 d later, the spleens of these $F_1$ animals were removed and pooled, and a single-cell suspension was prepared. Cells were washed twice and cultured for 24 h in 5-ml amounts at a concentration of $5 \times 10^6$ cells/ml. The culture medium consisted of sterile Hepes-buffered RPMI-1640 supplemented with L-glutamine (2 mM), 2-mercaptoethanol ($2 \times 10^{-5}$ M), 10% fetal calf serum, streptomycin (50 U/ml), and penicillin (50 U/ml).

*Generation of $F_1$-activated Donor T Cells by Mixed Lymphocyte Reaction (MLR) In Vitro.* Spleen cells were obtained under aseptic conditions, washed twice in sterile Hepes-buffered RPMI-1640 that contained 10% fetal calf serum and resuspended in culture medium. Normal spleen cells from (C57BL/10 × DBA/2)$F_1$ hybrids were irradiated in vitro with 3,300 rad (667 rad/min) and served as stimulator cells. T cell-enriched spleen cells were used as responder cells. Responder cells ($12 \times 10^6$) and stimulator cells ($12 \times 10^6$) were incubated in 5 ml medium for 5 d at 37°C in humidified air containing 5% $CO_2$.

*Cell-mediated Lymphocytotoxicity (CML) Assay.* The CML activity of activated donor T cells and the inhibition of CML activity with unlabeled target cells were determined and calculated as described elsewhere (13).

*Incorporation of $^{59}Fe$ and 5-[$^{125}I$]Iodo-Deoxyuridine ([$^{125}I$]UdR) into Lymphohemopoietic Organs.* The $F_1$ mice received drinking water containing 0.1% KI during 24 h before injection of isotopes. They were injected i.v. with a mixture of 0.2 μCi [$^{59}Fe$]citrate and 0.5 μCi $^{125}I$-UdR (catalogue No. IFS 2P, 1.M.355; Amersham Corp., Arlington Heights, Ill.). 18 h later, the spleens, thymuses, and one femur plus tibia were removed from the mice, and the $^{59}Fe$ and $^{125}I$ uptakes were measured in a gamma counter. The $^{125}I$ uptake of the thymuses was corrected for the emission by $^{59}Fe$.

*IgG Production Assay.* This assay served to determine the amount of IgG released into the culture supernate by cultured spleen cells from GVH $F_1$ mice. Single-cell suspensions were prepared from the spleens of individual recipient mice in Hepes-buffered RPMI-1640. Erythrocytes were lysed with a bicarbonate-buffered $NH_4Cl$ solution (0.155 M $NH_4Cl$, 0.01 M $KHCO_3$, 0.1 mM EDTA; pH 7.4). After the cells had been washed in Hepes-buffered RPMI-1640, 1-ml aliquots containing $25 \times 10^6$ live cells were cultured in RPMI-1640 that contained Hepes (10 mM), $NaHCO_3$ (12 mM), glutamine (2 mM), fetal calf serum (10%), penicillin (50 U/ml), and streptomycin (50 U/ml). The cells were cultured for 18 h at 37°C. The IgG concentrations in the culture supernates were determined by a solid-phase radioimmunoassay specific for mouse IgG.

*Radioimmunoassay for the Quantitation of Mouse IgG in Supernates of Cultured Spleen Cells.* The principle of the assay was a slight modification of the solid-phase double-sandwich technique described by Habermann (14). The solid-phase consisted of rabbit anti-mouse IgG coupled to Sepharose 4B (Pharmacia Fine Chemicals, Uppsala, Sweden). Mouse IgG from the test specimen after having reacted with this was detected by the use of $^{125}I$-labeled anti-mouse IgG. The same rabbit anti-mouse antiserum was used for the solid-phase and $^{125}I$-labeling; it was prepared by immunization of rabbits with the Fc fragments of polyclonal mouse IgG. Applying pure myeloma mouse IgG (catalogue No. 8402-24, Bionetics, Kensington, England) to obtain a reference curve, the IgG concentration in the test samples could be determined by comparison. IgG concentrations of 100–3,000 ng/ml could be quantitated. The test failed to detect myeloma IgM (TEPC 183; catalogue No. 8402-39, Bionetics) at a concentration of up to 10,000 ng/ml.

## Results

*Ability of B10 Strains and Inability of DBA Strains to Induce Lethal Runt Disease (Table I).* For the induction of lethal runt disease, groups of nonirradiated $F_1$ mice were injected i.v. with $60 \times 10^6$ donor spleen or lymph node cells. Whereas the vast majority of female (C57BL/10 × DBA/2)$F_1$ mice injected with spleen or lymph node cells from B10.D2 and C57BL/10 mice, respectively, died from runt disease, those $F_1$ mice injected with spleen or lymph node cells from DBA/2 survived and did not show even minor symptoms of runt disease. Injection of B10.D2 bone marrow cells, in contrast

TABLE I

*Comparative Survival of $F_1$ Recipients Injected i.v. with 60 × $10^6$ Lymphoid Cells of Different Origin*

| Donor strain | | | $F_1$ hybrid recipients | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Spleen cells | Lymph node cells | BMC | Strain | H-2 incompatibility | Number | Survivors at week indicated | | | | | | |
| | | | | | | 2 | 4 | 6 | 8 | 10 | 12 | 14 |
| | | | | | | % | | | | | | |
| B10.D2 | | | (B10.D2 × DBA/2)$F_1$ | None | 12 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| DBA/2 | | | (C57BL/10 × DBA/2)$F_1$ | H-$2^b$ | 20 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| | DBA/2 | | (C57BL/10 × DBA/2)$F_1$ | H-$2^b$ | 12 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| C57BL/10 | | | (C57BL/10 × DBA/2)$F_1$ | H-$2^d$ | 29 | 100 | 66 | 28 | 24 | 21 | 10 | 3 |
| B10.D2 | | | (C57BL/10 × DBA/2)$F_1$ | H-$2^b$ | 20 | 100 | 60 | 40 | 20 | 15 | 15 | 5 |
| | B10.D2 | | (C57BL/10 × DBA/2)$F_1$ | H-$2^b$ | 33 | 100 | 18 | 12 | 12 | 12 | 12 | 12 |
| | | B10.D2 | (C57BL/10 × DBA/2)$F_1$ | H-$2^b$ | 10 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| DBA/2 | | | (B10.M × DBA/2)$F_1$ | H-$2^f$ | 16 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| B10.D2 | | | (B10.M × DBA/2)$F_1$ | H-$2^f$ | 29 | 100 | 30 | 17 | 14 | 14 | 14 | 14 |
| DBA/2 | | | (B10.BR × DBA/2)$F_1$ | H-$2^k$ | 16 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| B10.D2 | | | (B10.BR × DBA/2)$F_1$ | H-$2^k$ | 19 | 100 | 44 | 16 | 11 | 11 | 0 | 0 |
| B10.BR | | | (B10.BR × DBA/2)$F_1$ | H-$2^d$ | 20 | 100 | 89 | 68 | 47 | 36 | 31 | 31 |
| B10.A | | | (B10.BR × DBA/2)$F_1$ | H-$2^{k/d}$* | 39 | 100 | 30 | 3 | 3 | 3 | 3 | 3 |
| DBA/2 | | | (B10.G × DBA/2)$F_1$ | H-$2^q$ | 15 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| B10.D2 | | | (B10.G × DBA/2)$F_1$ | H-$2^q$ | 11 | 100 | 64 | 18 | 18 | 18 | 9 | 9 |
| B10.G | | | (B10.G × DBA/2)$F_1$ | H-$2^d$ | 17 | 100 | 0 | 0 | 0 | 0 | 0 | 0 |
| DBA/1 | | | (B10.G × DBA/2)$F_1$ | H-$2^d$ | 15 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| DBA/2 | | | (B10.S × DBA/2)$F_1$ | H-$2^s$ | 18 | 100 | 100 | 100 | 100 | 100 | 100 | 100 |
| B10.D2 | | | (B10.S × DBA/2)$F_1$ | H-$2^s$ | 13 | 100 | 92 | 85 | 85 | 85 | 77 | 77 |

* The H-2 incompatibility between B10.A and (B10.BR × DBA/2)$F_1$ consists of the d haplotype at the left-hand side (K through I-E) and of the k haplotype on the right-hand side (I-C through D).

with that of spleen or lymph node cells, failed to induce lethal runt disease. When male (C57BL/10 × DBA/2)$F_1$ hybrids were used as recipients of spleen cells from either male C57BL/10 or male DBA/2 donors, we again observed that most of the C57BL/10-cell-injected, but none of the DBA/2-cell-injected, $F_1$ recipients died from lethal runt disease (data not shown).

The antigens that elicited the lethal reaction of donor T cells were determined by the H-2 complex of the $F_1$ recipients. This is evident from the complete absence of GVH mortality in (B10.D2 × DBA/2)$F_1$ recipients (H-$2^{d/d}$) injected with B10.D2 (H-$2^{d/d}$) spleen cells. The only difference between these $F_1$ hybrids and (C57BL/10 × DBA/2)$F_1$ hybrids (H-$2^{b/d}$) resides in the H-2 complex.

In the presence of an H-2 incompatibility in the $F_1$ recipient strains and irrespective of the H-2 incompatibilities involved, the injection of B10.D2 spleen cells consistently induced lethal runt disease. All the other H-2-congenic-resistant donor strains on a B10 background, namely B10.BR, B10.A, and B10.G, likewise induced lethal runt disease when injected into the corresponding H-2-incompatible $F_1$ hybrids. If an H-2 incompatibility existed between the recipients and donor spleen cells that were administered, the only mice that did not develop lethal runt disease were those that received DBA/2 or DBA/1 cells.

Although the GVH reactivity of the five H-2-congenic resistant B10 donor strains was fairly uniform in that all of them were capable of indúcing lethal runt disease, there were differences in the extent to which this occurred (Table I). Possibly, these quantitative differences are caused by the different H-2 alleles of the donor and $F_1$ strains involved.

*A Recessive Non-H-2 Locus Codes for the Capacity of B10.D2 to Induce Lethal Runt Disease (Table II).* (C57BL/10 × DBA/2)$F_1$ hybrids were injected i.v. with 60 × $10^6$ donor

TABLE II

*Comparison of Individual Donor Mice of Different Origins with Respect to Their Capacity to Induce Lethal Runt Disease in (C57BL/10 × DBA/2)F1 Hybrids*

| Donor mice (H-$2^{d/d}$) | $F_1$ hybrid recipients (H-$2^{b/d}$)* | | | |
|---|---|---|---|---|
| | Number tested | Survivors at week indicated | | |
| | | 4 | 8 | 14 |
| | | | % | |
| (B10.D2 × DBA/2)$F_1$ | 17 | 100 | 100 | 100 |
| B10.D2 | 17 | 70 | 30 | 11 |
| Backcrosses | 47 | 93 | 68 | 47 |

* Each $F_1$ hybrid recipient received an i.v. injection of 60 × $10^6$ live spleen cells obtained from an individual donor mouse.

spleen cells of individual donors. The following kinds of donor were used: (B10.D2 × DBA/2)$F_1$, [(B10.D2 × DBA/2)$F_1$ × B10.D2] backcross, and B10.D2 mice. None of the recipients of (B10.D2 × DBA/2)$F_1$ spleen cells died from runt disease, indicating that the runt disease-inducing capacity of B10.D2 is recessive to the noninducing capacity of DBA/2. As expected, most (89%) of the recipients of B10.D2 spleen cells died from runt disease. When spleen cells from 47 individual [(B10.D2 × DBA/2)$F_1$ × B10.D2] backcross mice were used as donor-cell inocula, 53% of the $F_1$ recipients died from runt disease. Statistical analysis of the numbers of $F_1$ hybrids that were dead at 14 wk after the induction of GVHR was performed by applying the binomial test. We examined the probabilities that the percentage of runted $F_1$ recipients of spleen cells from individual backcross donors were 50 and 25%, respectively, of the percentage of runted $F_1$ recipients of spleen cells from individual B10.D2 donors. The hypothesis that this value is 50% was not rejected ($P = 0.27$), whereas the hypothesis that it is 25% was clearly rejected ($P = 0.001$). These observations indicate that the difference in the runt disease-inducing capacity between strains B10.D2 and DBA/2 is determined by one genetic locus, or a number of closely linked loci.

*Capacity of B10 Donor Strains to Induce Lethal GVHD Is Associated with Their Capacity to Induce Aplastic Anemia and Thymic Hypoplasia (Tables III and IV).* Injections of 60 × $10^6$ spleen cells from C57BL/10 or B10.D2 into (C57BL/10 × DBA/2)$F_1$ recipients strongly suppressed the $^{59}$Fe incorporation into bone marrow of the recipients, whereas the injection of DBA/2 spleen cells did not. In contrast, the incorporation of $^{59}$Fe into the spleen was increased, especially if C57BL/10 or B10.D2 spleen cells were injected (Table III). Nevertheless, the net effect of the injection of spleen cells from C57BL/10 or B10.D2 on hemopoiesis was a severe decrease of the hematocrit values of the $F_1$ recipients. Such a decrease was not observed after the injection of spleen cells from DBA/2.

The strongly suppressive effects upon hemopoiesis in the bone marrow of the $F_1$ recipients were not observed when 60 × $10^6$ B10.D2 BMC instead of spleen cells were administered. This suggests that mature donor T cells were required for the induction of hemopoiesis suppression. When 60 × $10^6$ B10.D2 (H-$2^{d/d}$) spleen cells were injected into (B10.D2 × DBA/2)$F_1$ hybrids (H-$2^{d/d}$), the suppression of hemopoiesis in the

TABLE III

*Comparison of the Effect of Spleen Cells and BMC from Different Donor Strains upon the Hemopoiesis of $F_1$ Hybrid Recipients*

| Exp. | Donor cells | $F_1$ hybrid recipients* | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Strain | H-2 incompatibility | $^{59}$Fe incorporation into | | | | Hematocrit values | |
| | | | | Bone marrow | | Spleen | | Week 3 | Week 4 |
| | | | | Week 3 | Week 4 | Week 3 | Week 4 | | |
| 1 | Spleen cells (C57BL/10 × DBA/2)$F_1$ | (C57BL/10 × DBA/2)$F_1$ | None | 100 ± 6‡ | 100 ± 4 | 100 ± 14 | 100 ± 11 | 41.5 ± 0.3 | 46.6 ± 0.5 |
| | DBA/2 | (C57BL/10 × DBA/2)$F_1$ | H-$2^b$ | 80 ± 9§ | 89 ± 6‖ | 127 ± 18‖ | 182 ± 21¶ | 45.5 ± 0.7¶ | 42.4 ± 0.7¶ |
| | C57BL/10 | (C57BL/10 × DBA/2)$F_1$ | H-$2^d$ | 23 ± 2¶ | 38 ± 9¶ | 240 ± 27¶ | 233 ± 31¶ | 27.0 ± 0.4¶ | 37.6 ± 1.4¶ |
| | B10.D2 | (C57BL/10 × DBA/2)$F_1$ | H-$2^b$ | 36 ± 6¶ | 42 ± 4¶ | 225 ± 12¶ | 276 ± 28¶ | 27.5 ± 2.3¶ | 36.3 ± 0.8¶ |
| 2 | Spleen cells (B10.D2 × DBA/2)$F_1$ | (B10.D2 × DBA/2)$F_1$ | None | 100 ± 9 | 100 ± 7 | 100 ± 13 | 100 ± 23 | 45.4 ± 0.5 | 44.4 ± 0.5 |
| | B10.D2 | (B10.D2 × DBA/2)$F_1$ | None | 92 ± 6‖ | 69 ± 10‖ | 99 ± 16‖ | 201 ± 31§ | 45.7 ± 0.6‖ | 43.2 ± 1.0‖ |
| 3 | BMC (C57BL/10 × DBA/2)$F_1$ | (C57BL/10 × DBA/2)$F_1$ | None | 100 ± 8 | 100 ± 4 | 100 ± 17 | 100 ± 17 | 47.2 ± 0.5 | 47.8 ± 0.2 |
| | B10.D2 | (C57BL/10 × DBA/2)$F_1$ | H-$2^b$ | 93 ± 10‖ | 79 ± 8§ | 83 ± 24‖ | 122 ± 5‖ | 47.6 ± 0.7‖ | 47.2 ± 0.4‖ |

* Groups of mice were injected with $60 \times 10^6$ cells of the indicated donor strain: experiment 1 consisted of groups of 9–11 mice; experiments 2 and 3 consisted of groups of 4–6 mice.

‡ Mean ± SE expressed as percentage of the values found in syngeneically injected $F_1$ hybrids. Statistical analyses were performed by Student's $t$ test.

§ $0.01 < P < 0.05$ when compared with the values found in syngeneically injected $F_1$ hybrids.

‖ $P > 0.05$.

¶ $P < 0.01$.

bone marrow was much less severe than after injection of these cells into the H-2-incompatible (C57BL/10 × DBA/2)$F_1$ hybrids (H-$2^{b/d}$) (Table III). Because the two kinds of $F_1$ recipient were identical except for their H-2 alleles, we can conclude that an H-2 difference between donor and host was required for a profound depression of hemopoiesis.

Thymic hypoplasia was quantitated by the incorporation of [$^{125}$I]UdR into the thymuses of (C57BL/10 × DBA/2)$F_1$ recipients. Injection of $60 \times 10^6$ spleen cells from both C57BL/10 and B10.D2 led to a severe suppression of thymic [$^{125}$I]UdR incorporation at both weeks 3 and 4 (Table IV). By contrast, injection of $60 \times 10^6$ DBA/2 spleen cells failed to induce a significant suppression at week 3 and even led to a moderate stimulation of thymic cell proliferation at week 4 (Table IV).

Thymic hypoplasia was not observed when $60 \times 10^6$ B10.D2 BMC instead of spleen cells were injected into (C57BL/10 × DBA/2)$F_1$ hybrids, suggesting that donor T cells were needed to induce thymic hypoplasia. When $60 \times 10^6$ B10.D2 spleen cells were injected into (B10.D2 × DBA/2)$F_1$ hybrids, no significant decrease of thymic cell proliferation was observed (Table IV), indicating that an H-2 difference between donor cells and recipients is necessary for the induction of thymic hypoplasia.

*Capacity of Donor Strains to Induce Lethal GVHD Is Associated with the Capacity to Suppress Splenic IgG Production in the $F_1$ Hybrid Recipients (Table V).* Several $F_1$ hybrid strains were prepared by mating various H-2-congenic resistant B10 strains with DBA/2. Mice of these $F_1$ strains were injected with spleen cells from either DBA/2 or a B10 strain (Table V). After 14 d, the spleen cells of the $F_1$ recipients were assayed in vitro

TABLE IV

*Comparison of the Effect of Spleen Cells and BMC Obtained from Various Donor Strains upon the Thymic Uptake of [$^{125}$I]UdR of GVH $F_1$ Recipients*

| Exp. | Donor cells | $F_1$ hybrid recipients* | | | |
|---|---|---|---|---|---|
| | | Strain | H-2 incompatibility | [$^{125}$I]UdR uptake of thymuses | |
| | | | | Week 3 | Week 4 |
| 1 | Spleen cells | | | | |
| | (C57BL/10 × DBA/2)$F_1$ | (C57BL/10 × DBA/2)$F_1$ | None | 100 ± 10‡ | 100 ± 9 |
| | DBA/2 | (C57BL/10 × DBA/2)$F_1$ | H-2$^b$ | 83 ± 13§ | 124 ± 14§ |
| | C57BL/10 | (C57BL/10 × DBA/2)$F_1$ | H-2$^d$ | 18 ± 4‖ | 28 ± 5‖ |
| | B10.D2 | (C57BL/10 × DBA/2)$F_1$ | H-2$^b$ | 9 ± 0.3‖ | 23 ± 4‖ |
| 2 | Spleen cells | | | | |
| | (B10.D2 × DBA/2)$F_1$ | (B10.D2 × DBA/2)$F_1$ | None | 100 ± 9 | 100 ± 13 |
| | B10.D2 | (B10.D2 × DBA/2)$F_1$ | None | 95 ± 6§ | 88 ± 11§ |
| 3 | BMC | | | | |
| | (C57BL/10 × DBA/2)$F_1$ | (C57BL/10 × DBA/2)$F_1$ | None | 100 ± 19 | 100 ± 11 |
| | B10.D2 | (C57BL/10 × DBA/2)$F_1$ | H-2$^d$ | 77 ± 13§ | 96 ± 20§ |

* Same GVH mice as in Table III.

‡ Mean ± SE expressed as percentage of the values found in syngeneically injected $F_1$ hybrids. Statistical analysis was performed by Student's $t$ test.

§ $P > 0.05$ when compared with the values found in syngeneically injected $F_1$ hybrids.

‖ $P < 0.01$.

TABLE V

*Comparison of the Effect of Spleen Cells Obtained from Various Donor Strains upon the Production of IgG by Cultured Spleen Cells of GVH $F_1$ Recipients*

| Donor strain for induction of GVHR | $F_1$ hybrid recipients* | | | | | | |
|---|---|---|---|---|---|---|---|
| | Strain | H-2 incompatibility | IgG in supernates of cultured spleen cells obtained from $F_1$ mice injected with the stated number (× 10$^6$) of donor spleen cells | | | | |
| | | | 0 | 10 | 30 | 100 | 300 |
| (C57BL/10 × DBA/2)$F_1$ | (C57BL/10 × DBA/2)$F_1$ | None | 100 ± 21‡ | NT§ | 97 ± 17‖ | 97 ± 24‖ | 107 ± 5‖ |
| C57BL/10 | (C57BL/10 × DBA/2)$F_1$ | H-2$^d$ | 100 ± 24 | 193 ± 39¶ | 292 ± 63** | 30 ± 9** | 24 ± 2¶ |
| DBA/2 | (C57BL/10 × DBA/2)$F_1$ | H-2$^b$ | 100 ± 29 | 341 ± 126¶ | 511 ± 104** | 5,003 ± 1,083** | 7,530 ± 885** |
| B10.D2 | (C57BL/10 × DBA/2)$F_1$ | H-2$^b$ | 100 ± 22 | 45 ± 11¶ | 28 ± 8** | 122 ± 36‖ | 46 ± 8¶ |
| B10.BR | (B10.BR × DBA/2)$F_1$ | H-2$^d$ | 100 ± 13 | 128 ± 31‖ | 81 ± 19‖ | 20 ± 3** | NT§ |
| DBA/2 | (B10.BR × DBA/2)$F_1$ | H-2$^k$ | 100 ± 36 | 313 ± 76¶ | 553 ± 96** | 188 ± 35‖ | 1,175 ± 359** |
| DBA/2 | (B10.S × DBA/2)$F_1$ | H-2$^s$ | 100 ± 21 | 223 ± 36¶ | 689 ± 146** | 102 ± 17‖ | 1,965 ± 289** |
| DBA/2 | (B10.G × DBA/2)$F_1$ | H-2$^q$ | 100 ± 18 | 139 ± 25‖ | 255 ± 63¶ | 807 ± 261** | 761 ± 424‖ |
| DBA/2 | (B10.M × DBA/2)$F_1$ | H-2$^f$ | 100 ± 22 | 148 ± 24‖ | 605 ± 145** | 211 ± 72‖ | 3,511 ± 993** |

* Groups of five $F_1$ recipient mice were injected either i.v. with 10, 30, and 100 × 10$^6$ donor spleen cells or i.p. with 300 × 10$^6$ donor spleen cells per recipient. After 14 d, 25 × 10$^6$ recipient spleen cells were cultured and the IgG concentration in the culture supernates was determined.

‡ Expressed as a percentage of the IgG concentration found in the culture supernates of spleen cells from noninjected syngeneic $F_1$ mice (mean ± SE). Statistical analysis was performed by Student $t$ test.

§ Not tested.

‖ $P > 0.05$ when compared with the values found in noninjected syngeneic $F_1$ mice.

¶ $0.01 < P < 0.05$.

** $P < 0.01$.

for the amount of IgG released. Although spleen cells of the donor DBA/2 consistently failed to suppress and, in most instances, induced a marked stimulation of the IgG production of the spleen cells of the recipients, the B10 donor strains induced a marked suppression of the IgG production that was most profound after the injection of high doses of donor spleen cells. The induction of markedly increased IgG production after the injection of DBA/2 cells depended on the presence of T lymphocytes in the donor inoculum and on an H-2 incompatibility in the $F_1$ hybrid recipients (E. H. van Elven, unpublished results).

The suppression of IgG production in the spleen cells of the recipients likewise depended on the injection of donor T cells. Although C57BL/10 donor spleen cells that had been treated with normal mouse serum and C induced a marked suppression of IgG production in the $F_1$ recipients, the same number of those C57BL/10 spleen cells that remained alive after treatment with anti-Thy-1.2 and C did not (Table VI).

*Inability of Strain DBA/2 to Induce Lethal Runt Disease is Not a Result of an Inability to Generate Anti-$F_1$ Killer T ($T^K$) cells.* T cell-enriched spleen cells of the donor strains C57BL/10, B10.D2, and DBA/2 were activated against $F_1$ antigens in 750-rad-irradiated (C57BL/10 × DBA/2)$F_1$ hybrids, cultured for 1 d, and subsequently tested by CML on $F_1$ target cells. Fig. 1 shows that virtually no difference in cytotoxic activity could be detected among the three strains. Similarly, when T cell-enriched spleen cells of the same donor strains were first activated against $F_1$ by MLR (applying a responder:stimulator cell ratio of 1:1) and then assayed by CML on $F_1$ target cells, hardly any difference in cytotoxic anti-$F_1$ activity could be detected (Fig. 2). The cytotoxic cells generated in this way were completely destroyed by treatment of the activated cells with anti-Thy-1.2 and complement; in one experiment, a reduction of the specific lysis from 30 to 2% was observed. Cold-target inhibition experiments with both in vivo and in vitro activated donor spleen cells indicated that the cytotoxic activity of $T^K$ cells from DBA/2 and B10.D2 was specifically directed against the opposite H-2 of the $F_1$ target cells (Table VII).

## Discussion

Although differences in the GVHR-inducing capacities of different inbred mouse strains have previously been reported (5, 7, 15, 16), the present data represent the first

TABLE VI

*T Lymphocytes of the C57BL/10 Donor Are Required for the Suppression of Splenic IgG Production in $F_1$ Hybrid Recipients*

| C57BL/10 donor spleen cells* | Splenic IgG production in $F_1$ recipients‡ |
|---|---|
| None | 100 ± 8 |
| 100 × $10^6$ treated with normal mouse serum and C | 14 ± 3§ |
| 100 × $10^6$ treated with anti-Thy-1.2 and C | 240 ± 72§ |

* Donor spleen cells were first treated as indicated and then the stated number of live cells was injected.

‡ Groups of five (C57BL/10 × DBA/2)$F_1$ hybrids were injected i.v. with 100 × $10^6$ live cells. After 14 d the $F_1$ spleen cells splenic IgG production was determined. The results are expressed and statistically analyzed as described in the legend of Table IV.

§ $P < 0.001$.

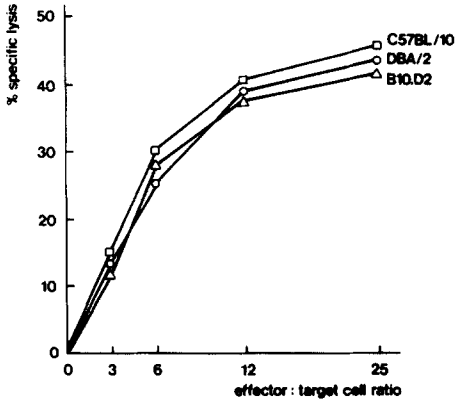


FIG. 1. Comparison of the anti-$F_1$ cytotoxic activity of T cells from strains C57BL/10, DBA/2, and B10.D2. T cell-enriched spleen cells from the respective donor strains were activated for 5 d in the spleens of irradiated (C57BL/10 × DBA/2)$F_1$ hybrids. Thereafter, they were assayed by CML by using $^{51}$Cr-labeled concanavalin A blast cells from syngeneic $F_1$ mice as target cells.

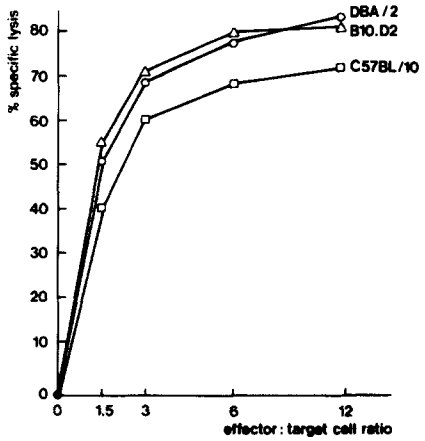


FIG. 2. Comparison of the anti-$F_1$ cytotoxic activity of T cells from strains C57BL/10, DBA/2, and B10.D2. T cell-enriched spleen cells from the donor strains were activated for 5 d in an MLR by irradiated spleen cells from (C57BL/10 × DBA/2)$F_1$. Thereafter, they were assayed by CML on $^{51}$Cr-labeled concanavalin A blast cells from syngeneic $F_1$ mice.

systematic multiparameter analysis of such differences. Our experiments were designed in such a way that lymphocytes obtained from several donor strains, strains DBA/2 and B10.D2 in particular, could be compared as donor cells for the induction of GVHR in groups of identical $F_1$ hybrid recipients. In contrast with the T cells of the B10 donor strains, those of strain DBA/2 consistently failed to induce lethal runt disease (Table I) or the symptoms associated with it (Tables III and IV). In previous studies, it has been observed that even doses much higher than $60 \times 10^6$ DBA/2 spleen and/or lymph node cells, likewise failed to induce lethal runt disease, but led to a chronic form of GVHD, whose hallmark was immune complex glomerulonephritis (5–7). However, it should be noticed that, as far as the induction of lethal GVHD by spleen cells from the B10 strains is concerned, the absolute number of cells required can vary (data not shown). In our experience, this variability was a result of the

TABLE VII

*Cold-Target Cell Inhibition of Genetically Different $T^K$ Cells Activated Against Antigens of (C57BL/10 × DBA/2)$F_1$ Hybrids*

| Effector cells* | | Cold-target inhibition‡ with cells from | |
|---|---|---|---|
| Strain | Mode of activation | (B10.D2 × DBA/2)$F_1$ (H-$2^{d/d}$) | C57BL/10 (H-$2^{b/b}$) |
| C57BL/10 (H-$2^{b/b}$) | In vivo | 97 ± 1 | 5 ± 2 |
| | In vitro | 96 ± 2 | 1 ± 1 |
| DBA/2 (H-$2^{d/d}$) | In vivo | 8 ± 2 | 98 ± 1 |
| | In vitro | 3 ± 1 | 92 ± 3 |
| B10.D2 (H-$2^{d/d}$) | In vivo | 10 ± 2 | 98 ± 1 |
| | In vitro | 2 ± 1 | 86 ± 3 |

* The activated effector cells were tested by CML with concanavalin A-stimulated, $^{51}$Cr-labeled (C57BL/10 × DBA/2)$F_1$ spleen cells as target cells, at an effector:target cell ratio 12.5:1. During CML, unlabeled (cold) target cells, either (B10.D2 × DBA/2)$F_1$ or C57BL/10 concanavalin A-stimulated spleen cells, were added at an unlabeled:labeled target cell ratio of 20:1.

‡ Inhibition ± SD was calculated as percentage of specific lysis obtained in control cultures to which no unlabeled target cells were added.

hygienic quality of the environment in which the animals were housed. This observation is consistent with the demonstration that the quality of the intestinal microflora exerts a tremendous influence on the mortality rate of mice undergoing GVHR (17).

A rather poor capability of strain DBA/2 to induce lethal GVHD has also been described in studies with parabiotic mice where a better survival was found in parabionts made between strains DBA/2 and, for instance, (DBA/2 × CBA)$F_1$ than in parabionts made between BALB/c and (BALB/c × CBA)$F_1$ (15, 18). However, this experimental design did not allow a comparison of the different parental strains in groups of genetically identical $F_1$ hybrids. Therefore, the possibility that the different survival rates observed with the various parental strains were also influenced by the different kinds of $F_1$ hybrids could not be excluded.

T lymphocytes of the donor are required for the induction of lethal runt disease by the GVHR (3, 4). In the present investigation, this was reflected by the finding that inocula of B10.D2 spleen or lymph-node cells killed the (C57BL/10 × DBA/2)$F_1$ recipients, whereas BMC completely failed to do so (Table I). Mouse spleens and lymph nodes contain 30–35 and 65–79% of T cells, respectively (19), but BMC comprise only ~2–7% of mature T cells (20).

The ability of strain B10.D2 and inability of strain DBA/2 to induce lethal runt disease was observed in all the H-2-incompatible $F_1$ strains tested (Table I). Although these two donor strains are H-2-identical and their T cells thus recognized the same H-2 incompatibilities in all recipients, only strain DBA/2 failed to kill the $F_1$ hybrids. Hence, both the ability of strain B10.D2 and the inability of strain DBA/2 to induce lethal runt disease were independent of the incompatible H-2 antigens offered to their T cells, i.e., H-$2^b$, H-$2^f$, H-$2^q$, or H-$2^s$. In other words, the two different kinds of GVH responsiveness observed with these two donor strains were not antigen specific.

Because strains DBA/2 and B10.D2 are H-2 identical, the locus or loci coding for their different capacities to induce lethal runt disease must be located outside of the

H-2 complex. This conclusion is consistent with our observation that all five H-2-congenic resistant B10 donor strains we used were able to induce lethal runt disease. A recessive mode of inheritance of strain capacity of B10.D2 mice to induce lethal runt disease in (C57BL/10 × DBA/2)$F_1$ recipients was indicated by the finding that (B10.D2 × DBA/2)$F_1$ donors failed to induce lethal runt disease in identical $F_1$ recipients. Moreover, we found that a single locus is responsible for the difference between nonkilling strains DBA/2 and (B10.D2 × DBA/2)$F_1$ on the one hand and the killing strain B10.D2 on the other (Table II). In conclusion, a recessively inherited non-H-2 locus (or a cluster of closely linked loci), the products of which are not specific for certain H-2 incompatibilities, accounts for the capability of B10.D2 T cells to induce lethal runt disease, by which capacity these T cells differ from those of strain DBA/2.

As death may result from other causes than GVHD, we compared the effects induced by different donor strains by quantitative parameters as well, such as the induction of aplastic anemia as a result of GVHD (1). It is known that, during the first weeks after the induction of runt disease, there is a marked suppression of erythropoiesis in the bone marrow, which may be partially compensated by a shift of erythropoiesis from the marrow to the spleen (21). In the present investigation, this pattern of anemia and disturbed hemopoiesis during acute GVHD was found after administration of donor spleen cells from both C57BL/10 and B10.D2, but not those from DBA/2 (Table III). Thus, by these parameters, too, T cells of strain DBA/2, in contrast with those of the B10 strains, proved to be incapable of inducing severe runt disease. Furthermore, we found that T cells obtained from both B10.D2 and C57BL/10 were capable of inducing the severe thymic hyoplasia of runt disease (1, 22), whereas T cells obtained from strain DBA/2 failed to do so (Table IV).

The inability of strain DBA/2 to induce lethal runt disease cannot be ascribed to a lack of T lymphocytes in the lymph nodes or spleen, because these organs contained closely similar percentages of Thy-1-positive cells in the three different donor strains DBA/2, B10.D2, and C57BL/10 (results not shown). Nor can the inability of strain DBA/2 mice to induce runt disease be ascribed to a general inability to induce a GVHR. This important conclusion is based on the finding that DBA/2 T cells were an excellent source of GVH-reactive donor cells when it came to the induction of autoantibody formation (11) and increased IgG production in $F_1$ mice (Table V). Interestingly, and in contrast with the injection of DBA/2 spleen cells, injection of high doses of spleen cells obtained from the strains on a B10 background induced a suppression of the splenic IgG production of the recipient (Table V). In all GVH assays used, an H-2 incompatibility in the nonirradiated recipients was required for induction of maximal abnormalities (Tables I, III, and IV).

*Pathogenesis of Lethal Runt Disease.* Because lethal runt disease kills the host, and because donor T cells are required for its induction, it has been assumed that runt disease is caused by donor $T^K$ cells (16, 23). Support for this assumption came from the observation that, in general, the capacities of different donor strains to induce runt disease correlated with their capacity in vitro to generate anti-recipient $T^K$ cells (24).

However, direct proof for the involvement of donor $T^K$ cells in the pathogenesis of lethal runt disease is missing. Although donor $T^K$ cells with anti-$F_1$ reactivity could readily be rescued from the lymphoid tissue of irradiated $F_1$ recipient mice (Fig. 1;

and [25–27]), up to now there are no reports that such T cells were detectable in nonirradiated $F_1$ hybrid recipients undergoing the GVHR. We found that strain DBA/2, although it was completely unable to induce runt disease, nevertheless was capable of generating $T^K$ cells specific for the incompatible H-2 antigens of the $F_1$ recipients (Figs. 1 and 2; and Table VII). Therefore, the genetic inability of strain DBA/2 to induce lethal runt disease cannot be ascribed to an intrinsic inability of this strain to generate anti-$F_1$ $T^K$ cells. However, because activated anti-$F_1$ $T^K$ cells of strain DBA/2 were studied only after short-term exposure to $F_1$ antigens either in vitro or in irradiated mice, we cannot exclude the possibility that, in the nonirradiated $F_1$ mice used for induction of lethal runt disease, the long-term activation of $T^K$ cells from strain DBA/2 might have been impaired. If that were so, inhibition of $T^K$ cells exclusively from strain DBA/2 would account for the inability of this strain to induce lethal runt disease.

Alternatively, a quality in which T cells from strain DBA/2 unmistakably differed from those of the B10 strains was their inability to induce the severe suppression of hemopoiesis in the bone marrow, lymphocyte proliferation in the thymus, and IgG production in the spleen. Taken together, these findings raise the possibility that lethal runt disease is caused not, or not only, by donor $T^K$ cells, but by those donor T cells that generate or mediate suppression of the physiological cell proliferation in lymphohemopoietic tissues (28–31). The following four observations are consistent with this possibility:

First, in contrast with donor $T^K$ cells with anti-$F_1$ specificity, suppressor T cells of the donor strain, which account for the general suppression of immune responsiveness during the early GVHR, were readily demonstrable in nonirradiated GVH $F_1$ mice (31, 33).

Second, Shand (31) has provided suggestive evidence that, after the initial specific triggering of parental-strain T cells by the incompatible H-2 antigens of the recipient, soluble factors were released that nonspecifically suppressed the formation of plaque-forming cells by innocent bystander cells. His finding extended the previous observation that the immunosuppression of GVH $F_1$ animals is nonspecific, because it affected not only lymphocytes of the H-2-incompatible $F_1$ host, but primed spleen cells of the parental donor strain as well (32).

Third, a nonspecific component is also involved in the suppression of hemopoiesis caused by the GVHR, because here, too, the suppression affected not only erythropoietic cells of the $F_1$ hybrid recipients but also those of the parental donor strain used for induction of the GVHR (34). In this type of experiment, killing of donor strain B cells and hemopoietic cells, by $T^K$ cells of syngeneic donors can be excluded, because donor T cells are tolerant of syngeneic cells.

Fourth, a notable feature of runt disease is that it preferentially affects such rapidly dividing tissues as the lympho-hemopoietic tissue and the epithelial tissue of the gastrointestinal tract and the epidermis, where it induces hypoplasia (1, 2). If donor $T^K$ cells were the main effector cells causing runt disease, then it is difficult to understand why just these highly proliferating tissues, which have a tremendous capacity for repair, should suffer most. By contrast, if a nonspecific suppression of cell proliferation were involved in the pathogenesis of runt disease, its manifestation would be expected precisely in those tissues in which intactness depends on a high rate of cell renewal.

## Summary

When comparing, in a murine model, the kind of graft-versus-host (GVH) disease (GVHD) induced by the donor strain DBA/2 on the one hand and several H-2-congenic resistant B10 donor strains on the other, we found that strain DBA/2 was a universal nonkilling GVH donor for H-2-incompatible nonirradiated $F_1$ hybrid recipients. In this respect, DBA/2 T cells differed from those of the H-2-identical donor strain B10.D2 as well as those of other B10 donor strains. The inability of strain DBA/2 to kill by GVH reaction was not limited to certain H-2 incompatibilities in the $F_1$ recipients, but was nonspecific. The inability to kill is determined by a dominant locus not linked to H-2.

DBA/2 T cells were also incapable of inducing the severe suppression of hematocrit values, bone marrow erythropoiesis, thymic cell proliferation, and splenic IgG production in the $F_1$ recipients that was observed after the injection of T cells from the B10 strains. However, DBA/2 T cells, in contrast with those of the B10 donor strains, were vigorous stimulators of IgG production in H-2-incompatible $F_1$ hybrid recipients. Surprisingly, strain DBA/2 as well as the B10 donor strains had a good capacity to generate anti-$F_1$ $T^K$ cells.

Taken together, these findings raise the possibility that lethal GVHD disease is not caused, or not caused exclusively, by donor killer T cells, but by those donor T cells that directly or indirectly induce a suppression of cell proliferation in certain vital organs of the recipient.

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## References

1. Elkins, W. L. 1971. Cellular immunology and the pathogenesis of graft-versus-host reactions. *Prog. Allergy.* **15:**78.
2. Rappaport, H., A. Khalil, O. Halle-Pannenko, L. Pritchard, D. Dantchev, and G. Mathé. 1979. Histopathologic sequence of events in adult mice undergoing lethal graft-versus-host reaction developed across H-2 and/or non-H-2 histocompatibility barriers. *Am. J. Pathol.* **96:**121.
3. Korngold, R., and J. Sprent. 1978. Lethal graft-versus-host disease after bone marrow transplantation across minor histocompatibility barriers in mice. Prevention by removing mature T cells from marrow. *J. Exp. Med.* **148:**1687.
4. Sekiguchi, M., G. Fuji, K. Sudo, and K. Suzuki. 1977. Failure to produce graft-versus-host reaction with splenic cells from athymic nude mice. *Jpn. J. Exp. Med.* **47:**531.
5. Gleichmann, E., H. Gleichmann, and R. S. Schwartz. 1972. Immunologic induction of malignant lymphoma: genetic factors in the graft-versus-host model. *J. Natl. Cancer Inst.* **49:** 793.
6. Gleichmann, E., H. Gleichmann, and W. Wilke. 1976. Autoimmunization and lymphomagenesis in parent → $F_1$ combinations differing at the major histocompatibility complex: model for spontaneous disease caused by altered self-antigens? *Transplant. Rev.* **31:**156.
7. Gleichmann, H., E. Gleichmann, J. André-Schwartz, and R. S. Schwartz. 1972. Chronic allogeneic disease. III. Genetic requirements for the induction of glomerulonephritis. *J. Exp. Med.* **135:**516.
8. Fialkow, P. J., C. Gilchrist, and A. C. Allison. 1973. Autoimmunity in chronic graft-versus-host disease. *Clin. Exp. Immunol.* **13:**479.

9. Gleichmann, E., and H. Gleichmann. 1976. Diseases caused by reactions of T lymphocytes to incompatible structures of the major histocompatibility complex. I. Autoimmune hemolytic anemia. *Eur. J. Immunol.* **6**:899.
10. Lindholm, L., L. Rydberg, and O. Stannegård. 1973. Development of host plasma cells during graft-versus-host reactions in mice. *Eur. J. Immunol.* **3**:511.
11. Gleichmann, E., and H. Gleichmann. 1980. Spectrum of diseases caused by alloreactive T cells, mode of sensitization to the drug diphenylhydantoin, and possible role of SLE-typical self-antigens in B-cell triggering. *In* Immunoregulation and Autoimmunity. R. S. Krakauer and M. K. Cathcart, editors. Elsevier North-Holland, Inc., New York. 73.
12. Julius, M. H., E. Simpson, and L. A. Herzenberg. 1973. A rapid method for the isolation of functional thymus-derived murine lymphocytes. *Eur. J. Immunol.* **3**:645.
13. Rolink, T., K. Eichmann, and M. M. Simon. 1978. Detection of two allotype-(Ig-1)-linked minor histocompatibility loci by the use of H-2-restricted cytotoxic lymphocytes in congenic mice. *Immunogenetics.* **7**:321.
14. Habermann, E. 1970. Ein neues Prinzip zur quantitativen Bestimmung hoch-molekularer Antigene (Verknüpfungstest) und seine Anwendung auf Tetanustoxin, Serumalbumin, und Ovalbumin. *Z. Klin. Chem. Klin. Biochem.* **8**:51.
15. Drell, D. W., and T. G. Wegmann. 1979. Immunological and genetic requirements for the parental hemopoietic takeover reaction in adult, H-2-incompatible parent-$F_1$ hybrid parabiosis. *Immunogenetics.* **8**:221.
16. Elkins, W. L. 1979. Tolerance and the fate of the allogeneic bone-marrow chimaera. *In* Immunological Tolerance and Enhancement. F. P. Stuart and F. W. Fitch, editors. MTP Press Ltd., Lancaster, England. 1.
17. van Bekkum, D. W., J. Roodenburg, P. J. Heidt, and D. van der Waaij. 1974. Mitigation of secondary disease of allogeneic mouse radiation chimeras by modification of the intestinal microflora. *J. Natl. Cancer Inst.* **52**:401.
18. Drell, D. W., and T. G. Wegmann. 1976. The genetics of tolerance induction in histoincompatible parabiosed mice. *Transplant. Proc.* **8**:363.
19. Raff, M. C., and J. J. T. Owen. 1971. Thymus-derived lymphocytes: their distribution and role in the development of peripheral lymphoid tissues of the mouse. *Eur. J. Immunol.* **1**:27.
20. Farrar, J. J., B. E. Loughman, and A. A. Nordin. 1974. Lymphopoietic potential of bone-marrow cells from aged mice: comparison of the cellular constituents of bone marrow from young and aged mice. *J. Immunol.* **112**:1244.
21. Pinno, E., and G. O. Bain. 1969. Erythropoietic shifts in the graft-vs-host reaction. *Arch. Pathol.* **88**:284.
22. Seemayer, T. A., W. S. Lapp, and R. P. Bolande. 1977. Thymic involution in murine graft-versus-host reaction. *Am. J. Pathol.* **88**:119.
23. Klein, J. 1978. Antigens and receptors involved in bone-marrow transplantation. *Transplant. Proc.* **10**:5.
24. Elkins, W. L. 1976. Correlation of graft-versus-host mortality and positive CML assay in the mouse. *Transplant. Proc.* **8**:343.
25. Cerottini, J. C., and K. T. Brunner. 1974. Cell-mediated cytotoxicity allograft rejection, and tumor immunity. *Adv. Immunol.* **18**:67.
26. Perren, B., G. Schumann, R. H. Gisler, and P. Dukor. 1974. Dissociation of cytotoxicity and helper activity of irradiated thymus cells. *Transplantation (Baltimore).* **17**:392.
27. Sprent, J., and J. F. A. P. Miller. 1972. Interaction of thymus lymphocytes with histocompatible cells. III. Immunological characteristics of recirculating lymphocytes derived from activated thymus cells. *Cell. Immunol.* **3**:213.
28. Ascensâo, J., W. Kagan, M. Moore, R. Pahwa, J. Hansen, and R. A. Good. 1976. Aplastic anaemia: evidence for an immunological mechanism. *Lancet.* **I**:669.

29. Cantor, H., and D. K. Gershon. 1978. Immunological circuits: cellular composition. *Fed. Proc.* **38:**2058.
30. Hoffman, R., E. D. Zanjani, J. D. Lutton, R. Zalusky, and L. R. Wasserman. 1977. Suppression of erythroid-colony formation by lymphocytes from patients with aplastic anaemia. *N. Engl. J. Med.* **296:**10.
31. Shand, F. L. 1976. Analysis of immunosuppresion generated by the graft-versus-host reaction. II. Characterization of the suppressor cell and its mechanism of action. *Immunology.* **31:**943.
32. Lawrence, W., and M. Simonsen. 1967. The property of "strength" of histocompatibility antigens, and their ability to produce antigenic competition. *Transplantation (Baltimore).* **5:** 1304.
33. Pickel, K., and M. K. Hoffmann. 1977. Suppressor T cells arising in mice undergoing a graft-vs-host response. *J. Immunol.* **118:**653.
34. Davis, W. E., and L. J. Cole. 1967. Suppression of hemapoietic colony-forming units in mice by graft-versus-host reaction. *Transplantation (Baltimore).* **5:**60.