ACUTE LETHAL GRAFT-VERSUS-HOST REACTION INDUCED BY MAJOR HISTOCOMPATIBILITY COMPLEX CLASS II–REACTIVE T HELPER CELL CLONES

By PAUL V. LEHMANN, GEORG SCHUMM,* DAVID MOON, URSULA HURTENBACH, FIORENZA FALCIONI, SIMONE MULLER, AND ZOLTAN A. NAGY

*From Preclinical Research, Sandoz Ltd., 4002 Basel, Switzerland; and the *Institute of Pathology, Eberhard-Karls University, 7400 Tübingen, Federal Republic of Germany*

Injection of lymphoid cells into healthy semiallogeneic hosts or immunocompromised allogeneic recipients results in graft-vs.-host disease (GVHD)[1]. The course of GVHD can be acute or chronic, depending on genetic and environmental factors. Thus far, studies of the pathomechanisms involved in GVHD were based on transfer of whole lymphoid cell populations, or T cells enriched for the CD4 or CD8 subset into recipients differing from the donors at class I or class II MHC loci or both. These studies have indicated that class II MHC disparity and CD4 cells are required for the induction of a chronic GVHD (1–3). However, the requirements to elicit acute GVHD have remained controversial. In the case of unirradiated recipients, acute GVHD occurs only in certain strain combinations of class I + class II disparity after injection of large numbers ($\sim 10^8$) of lymphoid cells (2, 3). Single MHC disparities and T cell subsets reacting to the relevant disparate molecules result in acute lethal GVHD only in irradiated recipients (with or without bone marrow reconstitution; references 4–8). The conclusion from these studies is that lethal GVHD is mediated primarily by CD8 cells reacting to class I molecules of the recipient, and to a smaller extent also by CD4 cells recognizing disparate class II molecules. The effect of these two T cell subsets appears to be additive.

In an attempt to dissect the cellular requirements for GVHD, we have injected class II–reactive cloned T cells into recipients expressing the stimulating class II molecules. The clones were found to induce acute lethal graft-vs.-host reaction (GVHR) without contribution of the hosts' immune system. The pathogenicity of clones did not correlate with their in vitro cytolytic activity. In this experimental system, in vivo activation of the injected cloned cells appears to be the only requirement of triggering the disease, which is manifested in severe vascular leakage as a cause of death.

Materials and Methods

Mice. Mice of strains B10.A(2R), B10.A(4R), B10.AQR, B10.BR, B10.D2, B10.M, and B10.T(6R) were obtained from Olac Ltd. (Bicester, UK). BALB/c, BALB/c *nu/nu*, C57BL/6,

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[1] *Abbreviations used in this paper:* GVHD, graft-vs.-host disease; GVHR, graft-vs.-host reaction; VLS, vascular leak syndrome.

CBA, and DBA/2 mice were purchased from Iffa Credo (L'Arbresle, France); and AKR mice from Bomholtgard (Ry, Danemark). C3H/HeN and C3H/HeJ mice were from Charles River Wiga (Sulzfeld, FRG), and C3H nu/nu mice were from the Zentralinstitut für Versuchstiere (Hannover, FRG). Mast cell–deficient (B6/C3H w^x/w^v)F$_1$ and congeneic nondeficient (B6/C3H +/+)F$_1$ mice were kindly provided by Dr. W. Beamer (The Jackson Laboratory, Bar Harbor, ME). All mice were specific pathogen free, and were used when 2–3 mo old.

T Cell Clones.   Clones were isolated from MLR combinations B10.A(4R) anti-B10.A(2R) (4R anti-2R) and B10.T(6R) anti-B10.AQR (6R anti-AQR). The former combination generates I-E^k-specific response, and the latter induces I-A^k as well as I-E^k-specific T cells. After four cycles of weekly restimulation with irradiated stimulator cells and without exogenous IL-2, the cells were cloned by a limiting dilution (0.3 cells/well), in the presence of fresh stimulator cells and human rIL-2 (10 ng/ml; Sandoz Research Institute, Vienna, Austria). The culture medium was RPMI 1640 supplemented with 10% FCS, antibiotics, L-glutamine, and 2-ME. Both 4R and 6R clones were derived from two different cell lines, indicated either by numbers or letters in the designation of clones. For maintaining without much expansion, the clones (2 × 10^5 cells/ml) were stimulated once a month with irradiated stimulator spleen cells (2.5 × 10^6/ml) and rIL-2, and fed weekly with rIL-2 containing medium. To expand the clones to large numbers (up to 5 × 10^8), 1 wk after restimulation with irradiated cells, the medium was removed completely, and replaced by fresh IL-2 containing medium. 3–4 d later, in the log phase of growth, the cultures were split and diluted three to four times (cell concentration readjusted to 2 × 10^5/ml) in IL-2 medium. The expansion was repeated every 3–4 d for 2 wk. The medium with nonadherent T cells was then removed completely, and the harvested cells were used for experiments. Since the cells were semi-adherent at this phase of growth, the ones adherent to the flasks after harvesting were sufficient to grow up again to saturating cell numbers (∼10^6/ml) for another 2 wk. After 1 mo of expansion, the cells stopped growing, and required restimulation with fresh irradiated spleen cells and IL-2. Large scale cultures were performed in 500-ml flasks (Falcon Labware, Oxnard, CA) in 50-ml vol for restimulation and 100 ml for expansion. The culture flasks were changed after 3–4 wk.

In Vitro Assays of T Cell Clones.   The specificity of clones was tested in a standard proliferation assay against different stimulator cells (2 × 10^4 cloned cells and 5 × 10^5 irradiated spleen cells) cultured for 3 d with [^{3}H]thymidine incorporation as a readout (9). The clones were also tested for cytolytic activity on LPS blast target cells as described previously (10). Cell surface expression of L3T4 and Lyt-2 molecules was tested by immunofluorescence using a phycoerythrin-labeled anti-L3T4 and biotin-labeled anti-Lyt-2 mAb (Becton Dickinson & Co., Mountain View, CA). Surface staining was evaluated by a Facscan.

In Vivo Proliferation of T Cell Clones.   Cloned cells (10^7/mouse) were injected intravenously in 0.5 ml PBS into syngeneic and stimulator strain recipients. After 60 h, 0.5 mCi methyl-[^{3}H]thymidine was injected intravenously into each mouse. Before injection, the alcohol content of thymidine was allowed to evaporate in a laminar flow hood for 4 h. After 18 h, the animals were killed, and the lungs and liver removed. Each lung was washed three times in PBS in 50-ml tubes by shaking at 4°C for 2 d. After washing, the tubes were centrifuged, and the lungs and the pelleted cells from the fluid phase were recovered and dissolved in 5 ml Soluene-100 (Packard Instrument Co., Inc., Downers Grove, IL) at 37°C in glass vials (rocking for at least 24 h). The livers were minced, washed three times in 50 ml PBS by shaking at 4°C for 1 h, and dissolved in 10 ml of Soluene-100. 100-µl aliquots of dissolved organs were mixed with 3 ml scintillation fluid, and counted in the liquid scintillation counter.

Homing Assay.   Cloned cells (10^7/ml) were labeled with 25 µCi/ml ^{51}Cr for 1 h, washed three times, and injected intravenously (10^7 cells/mouse). 1 d later, mice were killed, dissected, and the organs counted. The percentage counts recovered from each organ were related to the sum of counts from the whole mouse taken as 100%. The latter value corresponded to 70–80% of injected radioactivity (11).

Exudation Assay.   3 d after intravenous injection of 10^7 cloned T cells, 0.5 µCi of ^{125}I human albumin (Amersham, Buckinghamshire, UK) was injected intravenously. 2 h later, the animals were exsanguinated, and radioactivity in individual organs was counted (12).

Results

Characterization of the T Cell Clones Used.   Clones derived from MLR combinations 4R anti-2R and 6R anti-AQR were tested for proliferation against a panel of H-2-different stimulator cells. The representative experiment in Table I demonstrates that the reactivity of all 4R clones and of one 6R clone (6Re) requires the k allele in the I-E region to be expressed by the stimulator cells. Accordingly, the response of these clones can be inhibited by anti-I-E mAb (data not shown). In contrast, clone 6R2 reacts to stimulator cells expressing the I-A^k molecule, and crossreacts to H-2^d and H-2^f stimulator cells. The response to H-2^d stimulator cells was inhibited by anti-I-A^d mAb (data not shown). These clones were also tested for cytolytic activity (Fig. 1). Four of the six clones exhibited cytotoxicity against LPS blast target cells. The killer activity mapped concordantly with the proliferative response. All six clones were Thy-1$^+$, L3T4$^+$, Lyt-2$^-$, as detected by immunofluorescence, and expressed message for IFN-γ, IL-2, and TNF-β, but not for IL-4 (data not shown). The latter characteristics classify the clones as Th1 (13).

MHC-specific Induction of Lethal GVHR by the T Cell Clones.   Different numbers of cloned cells were injected intravenously into unirradiated recipients of different H-2 haplotypes. As shown in Table II, cloned cells caused 100% mortality within 5 d in a dose-dependent manner, provided that the recipients expressed the stimulatory class II molecule. Moreover, clone 6R2 exhibited identical crossreactivities in vitro and in vivo (Tables I and II). In contrast, syngeneic recipients, and recipients whose MHC molecules were not recognized by the clones, survived without any sign of disease, even after injection of as many as 6 × 10^7 cells (data not shown). All clones were capable of inducing lethal GVHD irrespective of their cytolytic activity in vitro, rendering direct cytotoxicity as a mechanism of the disease unlikely.

2–3 d after injection of a lethal dose of cells, the animals started to exhibit the typical symptoms of acute GVHD, such as ruffled fur, hunched posture, tachypnoea, apathia, hypothermia, and very rapid weight loss. Death of all animals occurred on days 4 and 5. Sublethal cell doses of 3–5 × 10^6 cells also induced these

TABLE I

MHC Specificity of Proliferative Response by T Cell Clones

| Stimulator cells from strain	Alleles at H-2 loci				Proliferative response by T cell clones					
	K	A	E	D	4R9	4Rh	4R5	4Ra	6R3	6R2
					cpm					
B10.A(4R)	k	k	b	b	621	230	662	386	266	357.513*
B10.A(2R)	k	k	k	b	58.438	99.338	104.076	96.080	89.482	273.721
B10.T(6R)	q	q	q	d	1.356	508	808	472	547	4.000
B10.AQR	q	k	k	d	78.987	113.016	105.578	71.216	90.504	348.068
B10.D2	d	d	d	d	591	271	667	428	422	464.558
C57.Bl/6	b	b	b	b	986	824	831	712	875	3.264
B10.M	f	f	f	f	570	909	1.334	414	669	234.823

* Significant responses are underlined.
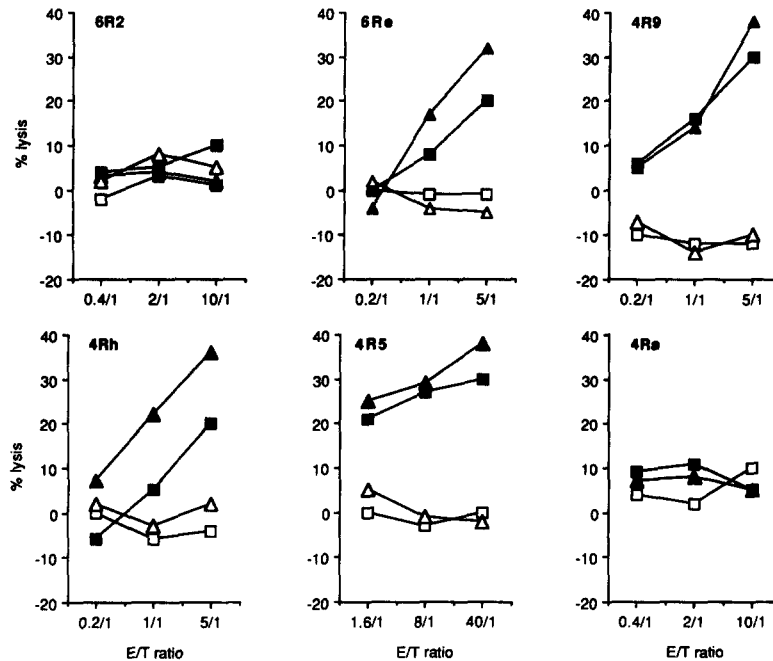
FIGURE 1.   Cytolytic activity of T cell clones. Clones were tested for cytotoxicity on ^{51}Cr-labeled LPS blast target cells from strains 2R (■); 4R (□); 6R (Δ); and AQR (▲).

symptoms, however, after day 5, the animals recovered and survived indefinitely. The survivors failed to exhibit signs of chronic GVHD (e.g., anti-DNA antibodies, elevated Ig levels, lymphoid cell infiltrations) within an observation period of 6 mo. Histological findings in the lethal syndrome were interstitial and alveolar oedema of lungs, dystelektasia, hyalin membranes in alveoli, and intravascular fibrin deposits. These findings are consistent with the diagnosis of shock. In the liver, focal mainly subcapsular necrosis was seen, a finding also consistent with shock.

Homing and Activation of T Cell Clones In Vivo.   As shown in Fig. 2, all clones homed preferentially to lungs and liver. After 24 h, 80–90% of total radioactivity was recovered from these two organs. The residual activity was distributed among spleen (~5%), kidneys (~3%), and skin (~3%). A slight preference in homing to the liver was seen in syngeneic mice and to the lungs in stimulator strain mice. We then investigated whether the cloned cells became activated at the sites of homing by using an in vivo [³H]thymidine incorporation assay. The data in Fig. 3 demonstrate a dramatically increased thymidine incorporation in the lungs of stimulator strain recipients, as compared with syngeneic animals. A similar although less clear-cut difference was measured also in the liver. As shown in Table III, despite irradiation of the hosts with 2,000 rad before injection of the cloned cells, 56% of the thymidine incorporation was still detectable. Thus, the majority of proliferative response appears to be contributed by the injected cells, although a host component is also present. Inhibition of DNA synthesis of the clone with mitomycin *C* before injection reduced the in vivo proliferation by 95%. Thus, the host component of thymidine incorporation

TABLE II

MHC Specificity and Cell Dose Dependence of Lethal GVHD Induced by T Cell Clones

Recipient strain	Alleles at H-2 loci				No. of cloned cells injected per animal	Mortality on day 5 after injection of clone					
	K	A	E	D		4R9	4Rh	4R5	4Ra	6Re	6R2
B10.A(4R)	k	k	b	b	10^7	0/5	0/4	0/3	0/3	0/3	5/5
B10.A(2R)	k	k	k	b	10^7	5/5	5/5	4/4	4/7*	3/3	3/3
					7.5×10^6	4/5	–‡	–	–	–	–
					5×10^6	2/8	2/5	–	–	–	–
					2.5×10^6	0/5	0/4	–	–	–	–
B10.T(6R)	q	q	q	d	10^7	0/3	0/3	0/3	0/3	0/3	0/4
B10.AQR	q	k	k	d	10^7	3/3	3/3	4/4	3/5*	10/10	6/6
					7.5×10^6	–	–	–	–	9/10	5/5
					5×10^6	–	–	–	–	4/8	2/5
					2.5×10^6	–	–	–	–	0/4	0/6
B10.D2	d	d	d	d	10^7	–	–	–	–	0/3	3/3
C57 Bl/6	b	b	b	b	10^7	–	–	–	–	0/3	0/3
B10.M	f	f	f	f	10^7	–	–	–	–	0/3	6/6

* 1.5×10^7 cells are required for 100% mortality.
‡ Not determined.

also seems to depend on activation of the injected cells. When the DNA synthesis was inhibited in both cloned cells and host, no increase in thymidine incorporation was observed. These results strongly suggest that the injected cells become activated and proliferate in stimulator strain hosts.

Vascular Leakage Induced by T Cell Clones in Stimulator Strain Recipients. The pathological findings raised the possibility that T cell activation might be accompanied by vascular leakage. This was investigated by extravasation of ^{125}I albumin 3 d after injection of cloned cells. The representative experiments in Fig. 4 demonstrate a highly increased vascular leakage in the lungs and, to some extent, also in the liver of stimulator strain mice, as compared with syngeneic animals. The vascular leakage in syngeneic mice did not significantly differ from that in mice without injected cells.

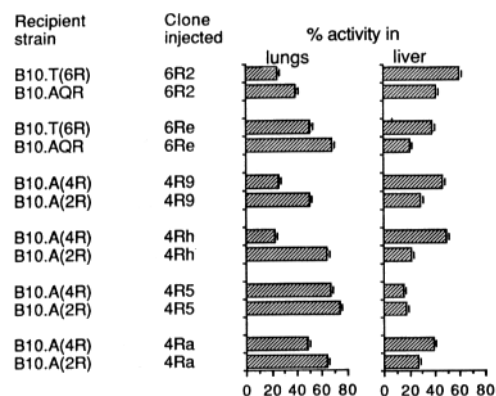*Acute Lethal GVHR Does not Require Host-derived Mechanisms.* Shock and vascular

FIGURE 2. Homing of cloned T cells in syngeneic and allogeneic recipients. ^{51}Cr-labeled cloned T cells (10^7/recipient) were injected intravenously. Percent activity was calculated as described in Materials and Methods. Each column represents the mean (and SD) for three mice.
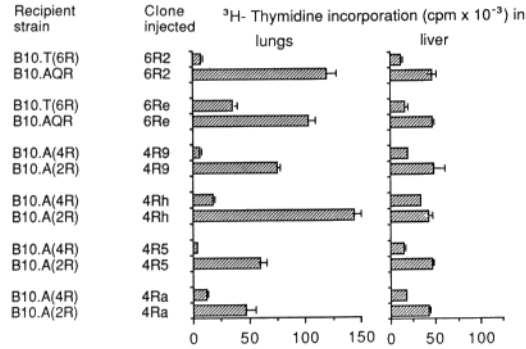
FIGURE 3. In vivo [³H]TdR incorporation induced by T cell clones in syngeneic and allogeneic recipients. 3 d after injection of cloned cells, recipient mice were given 0.5 mCi [³H]TdR, and the radioactivity in lungs and liver was determined as described in Materials and Methods. Each column represents the mean (and SD) for three mice.

leakage can result from several different inflammatory mechanisms. For example, TNF has been implicated in shock (14, 15), while mast cell degranulation (16, 17) and activated complement factors, in particular C5a (18), are known to increase vascular permeability. As shown in Table IV, mouse strains expressing the stimulatory class II molecule, and carrying genetic defects causing deficiency of C5, TNF-α, and mast cells, respectively, are as sensitive to the lethal disease as the nondeficient wild-type strains. Moreover, athymic nude mice were also sensitive to the disease and develop vascular leakage (Table V), indicating that the host's own T cell system plays little if any role in the pathogenesis of T cell clone–induced acute lethal GVHR. Inactivation of the complement system by cobra venom factor also failed to prevent lethal GVHR (Table VI). We also investigated whether or not antiinflammatory drugs with defined sites of action would interfere with the disease. The results are summarized in Table VII. Dexamethasone, an inhibitor of protease release from phagocytic cells (19), prostaglandin inhibitors indomethacin and acetylsalicylic acid, as well as Wortmannin that blocks the release of oxygen radicals from activated granulocytes and macrophages (20), were all ineffective in preventing or ameliorating

TABLE III

In Vivo [³H]TdR Incorporation Reflects Activation of the Injected T Cell Clone

Recipient		Clone injected		[³H]TdR incorporation in lungs
Strain	Treatment	Designation	Treatment	
	rad			*cpm*
B10.A(4R)	–	–	–	4,499 ± 770‡
B10.A(4R)	–	4R9	–	4,610 ± 1,131
B10.A(2R)	–	–	–	1,753 ± 248
B10.A(2R)	2,000	–	–	838 ± 153
B10.A(2R)	–	4R9	–	76,480 ± 3,339
B10.A(2R)	2,000	4R9	–	42,657 ± 1,375
B10.A(2R)	–	4R9	Mitoycin C§	3,618 ± 932
B10.A(2R)	2,000	4R9	Mitoycin C	1,046 ± 10

* 10⁷ cells/recipient.
‡ Mean + SD for three mice/group.
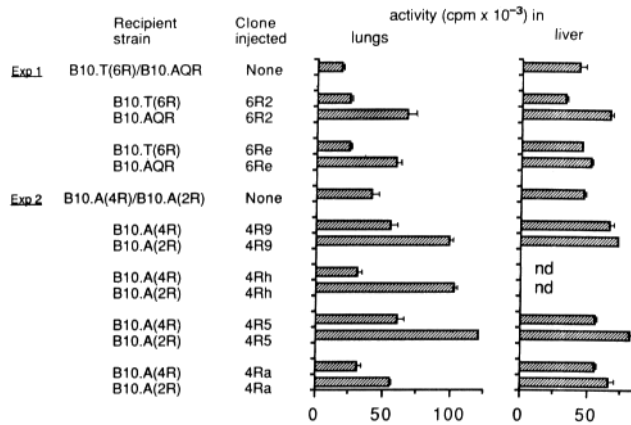§ Mitomycin C (50 μg/ml) for treating 10⁷/ml cells.

FIGURE 4. Extravasation of ^{125}I albumin induced by T cell clones. 3 d after injection of cloned cells ^{125}I human albumin was injected and the exudation assay performed as described (14). Each column represents the mean (and SD) for four mice.

the disease. Thus, secondary inflammatory reactions do not appear to be crucial for the pathogenesis of lethal GVHR. Of the drugs tested, only cyclosporin prevented the disease, probably by inhibiting activation of the injected cells themselves. Irradiated mice also developed the disease (data not shown). Collectively, the results argue against the involvement of host-derived mechanisms, and indicate by exclusion that the injected cloned cells directly induce vascular leakage and death.

Discussion

In this study, we have demonstrated that class II–specific Th1 cell clones can induce acute lethal GVHR in an antigen-specific manner upon injection into unir-

TABLE IV

Failure of Non-H-2 Background to Influence Lethal GVHD

Recipient strain			Mortality by day 5 after injection of 10^7 cells of clone	
Designation	H-2 haplotype	Genetic defect	4R9	6R2
B10.BR	k	–	2/2	3/3
AKR	k	C5	5/6	3/3
CBA	k	–	3/3	3/3
C3H/HeN	k	TNF-α	3/3	3/3
C3H/HeJ	k	–	5/5	3/3
C3H *nu/nu*	k	Athymic	4/6*	3/3
(B6xC3H +/+)F₁	b/k	–	3/3	2/3‡
(B6xC3H wˣ/wᵛ)F₁	b/k	Mast cells	3/3	3/3‡
B10.D2	d	–	–	3/3
DBA/2	d	C5	–	3/3
BALB/c	d	–	–	3/3
BALB/c *nu/nu*	d	Athymic	–	4/4§

* 1.2 × 10^7 of cloned cells were required for 100% mortality.
‡ 1.5 × 10^7 cells were injected.
§ 2 × 10^7 cells were required for 100% mortality.

TABLE V

T Cell Clone Induced Extravasation of ^{125}I Albumin in Nude Mice

| Recipient | Clone injected | | | Acitivity in lungs |
	Designation	Specificity	No.	
BALB/c nu/nu	–	–	–	50,997 ± 20,212*
BALB/c nu/nu	6Re	I-E^k	2 × 10^7	58,349 ± 27,542
BALB/c nu/nu	6R2	I-A^d	2 × 10^7	129,015 ± 17,893

* Mean ± SD for four mice/group.

radiated recipients. This disease exhibits similarities to, as well as differences from, the classical acute GVHD observed after injection of polyclonal lymphoid cell populations into semiallogeneic or irradiated hosts. The similarities include the nature of immunologic trigger, the clinical symptoms, and the rapid, therapy-resistant fatal course. However, the clone-induced disease has a more rapid course, which can be explained by the large number of injected antigen-primed cells, as compared with the low frequency of unprimed precursor T cells in polyclonal cell populations. Another difference is that the clone-induced disease is restricted to the lungs and liver due to preferential homing to these organs, whereas the classical disease involves the bone marrow, lymphatic system, liver, gastrointestinal tract, and the skin (21). It should be pointed out that preferential homing to lungs and liver is not only the property of in vitro derived clones (22, 23), but appears also to be the physiological consequence of in vivo T cell activation (24, 25), although in the latter case, localization of activated T cells to other tissues, such as the spleen and intestines, has also been reported (26). It is, therefore, unlikely that altered homing is the sole explanation for the differences between GVHD induced by Th1 clones and bulk lymphoid cells, respectively. Presumably, other T cell subclasses not represented by our clones, such as Th2 and CD8$^+$ cells, are responsible for additional features of the disease.

In agreement with previous studies (27, 28), antigen-specific activation of the cloned cells in vivo is a prerequisite for disease induction. This is reflected by increased

TABLE VI

Inactivation of the Complement System Fails to Prevent
Lethal GVHD by T Cell Clones

| Recipient strain | CVF* | Clone injected | | Mortality by day 5 |
		Designation	Cell no.	
	U			
B10.A(4R)	40‡	None	–	0/3
B10.A(4R)	40‡	6R2	10^7	4/4
B10.A(4R)	–	6R2	10^7	3/3
B10.BR	30§	None	–	0/2
B10.BR	30§	6Re	10^7	5/5
B10.BR	–	6Re	10^7	3/3

* Cobra Venom Factor from Naja naja kaouthia, intraperitoneally.
‡ 20 U 1 d before injection of clone, 10 U/d on the following 2 d.
§ 10 U/d starting 4 h before injection of the clone.

TABLE VII

Effect of Anti-inflammatory Drugs on Lethal GVHD Induced by T Cell Clones

Recipient strain	Drug	Dose	Route	Clone injected	Mortality by day 5
		mg/kg/d			
C3H/HeJ	Dexamethason	50	s.c.	–	0/3
C3H/HeJ	Dexamethason	50	s.c.	6R2, 8 × 10^6	4/4
C3H/HeJ	Dexamethason	10	s.c.	6R2, 8 × 10^6	3/4
C3H/HeJ	–	–	–	6R2, 8 × 10^6	3/3
B10.A(2R)	Dexamethason	50	s.c.	4R9, 8 × 10^6	4/4
B10.A(2R)	Dexamethason	10	s.c.	4R9, 8 × 10^6	3/3
B10.A(2R)	–	–	–	4R9, 8 × 10^6	3/3
C3H/HeJ	Indomethacin	10	p.o.	–	0/3
C3H/HeJ	Indomethacin	10	p.o.	6R2, 8 × 10^6	4/5
C3H/HeJ	Indomethacin	5	p.o.	6R2, 8 × 10^6	3/3
C3H/HeJ	–	–	–	6R2, 8 × 10^6	3/3
C3H/HeJ	Indomethacin	10	p.o.	4R9, 6 × 10^6	3/4
C3H/HeJ	Indomethacin	5	p.o.	4R9, 6 × 10^6	3/4
C3H/HeJ	–	–	–	4R9, 6 × 10^6	3/3
C3H/HeJ	ASA	500	p.o.	–	0/3
C3H/HeJ	ASA	500	p.o.	4R9, 10^7	4/4
C3H/HeJ	ASA	150	p.o.	4R9, 10^7	3/3
C3H/HeJ	–	–	–	4R9, 10^7	3/3
C3H/HeJ	Wortmannin*	3	p.o.	–	0/4
C3H/Hej	Wortmannin*	3	p.o.	4R9, 10^7	4/4
C3H/HeJ	Wortmannin*	1	p.o.	4R9, 10^7	4/4
C3H/HeJ	–	–	–	4R9, 10^7	3/3
C3H/HeJ	Wortmannin*	3	p.o.	6R2, 10^7	4/4
C3H/HeJ	Wortmannin*	3	p.o.	6R2, 10^7	4/4
C3H/HeJ	Cyclosporin	100	p.o.	–	0/4
C3H/HeJ	Cyclosporin	100	p.o.	6R2, 10^7	1/6
C3H/HeJ	Cyclosporin	50	p.o.	6R2, 10^7	0/8
C3H/HeJ	Cyclosporin	25	p.o.	6R2, 10^7	0/4
C3H/HeJ	–	–	p.o.	6R2, 10^7	3/3
B10.A(2R)	Cyclosporin	100	p.o.	4R9, 8 × 10^6	0/3
B10.A(2R)	Cyclosporin	50	p.o.	4R9, 8 × 10^6	0/3
B10.A(2R)	–	–	p.o.	4R9, 8 × 10^6	3/3

* Dissolved in 0.5% tragecanth.

thymidine incorporation in the inflicted organs. The thymidine is taken up to a large extent by the injected cells themselves, indicating proliferation of these cells in the host. However, the in vivo proliferative response possesses a distinct host component, whose nature is unknown. This component is clearly dependent on the recognition of the stimulating antigen in the host by the injected cloned cells. Possible candidates for proliferating host cells are, first, host lymphoid cells attracted and activated by lymphokines secreted by the injected cells; second, nonlymphoid cells, e.g., fibroblasts responding to clone-derived lymphokines; and third, host cells mounting an antiidiotypic response against the clone. The latter possibility is ren-

dered unlikely by the observations that mitomycin-treated cells fail to induce significant thymidine incorporation, and that this reaction is very pronounced already on day 3.

The in vivo activation of T cell clones is followed by a vascular leak syndrome (VLS) leading to death of the recipients. This syndrome has been previously described as a severe side effect of IL-2 therapy (29, 30). The latter type of VLS appeared to result from the activation of host lymphoid cells, particularly NK cells, by IL-2 (12, 31). Our study is the first to demonstrate that Th cell clones can induce VLS and lethal disease without the participation of several inflammatory mechanisms of the host, such as mast cell degranulation, generation of vasoactive or anaphylatoxic complement components, and release of proteases and oxygen radicals. By exclusion, therefore, the injected Th cells themselves are likely to induce vascular leakage directly. These results raise the possibility that the edema often accompanying T cell–dependent tissue reactions, such as delayed-type hypersensitivity, may also be directly triggered by T cells, and is not the consequence of secondary inflammatory mechanisms, as assumed previously (16). Thus, the capability of Th cells to induce vascular leakage can be considered as an effector mechanism, which facilitates the accumulation of inflammatory cells, and serum proteins at the site of pathogen invasion.

Summary

T cell clones isolated from class II MHC–disparate MLR combinations, and specific for I-A^k and I-E^k molecules, respectively, are shown to induce acute lethal graft-vs.-host disease in unirradiated recipients. Cytolytic and noncytolytic clones are equally efficient in this respect. The lethal disease is dependent on recognition of the stimulatory class II molecules in the host. The clones home to lungs and liver, and become activated in these organs as demonstrated by an in vivo thymidine incorporation assay. After activation, a severe vascular leak syndrome develops causing death of the recipients within 5 d after the injection of 5 × 10^6 to 10^7 cloned cells. The disease develops without the participation of secondary host–derived inflammatory mechanisms, such as mast cell degranulation, complement activation, and the release of prostaglandins, oxygen radicals, or proteolytic enzymes. The results raise the possibility that Th cells can directly influence vascular permeability, and control, thereby, the acute inflammatory reaction of blood vessels.

References

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