Gut Injury in Mouse Graft-Versus-Host Reaction

Study of Its Occurrence and Mechanisms

Delphine Guy-Grand and Pierre Vassalli

Institut National de la Santé et de la Recherche Medicale U.132, Groupe d'Immunologie et de Rhumatologie Pédiatriques, Hôpital Necker—Enfants Malades, 75730 Paris, France; and Department of Pathology, University of Geneva, 1211 Geneva 4, Switzerland

Abstract

The occurrence, nature, and pathogenesis of intestinal lesions were studied in a number of graft vs. host reaction (GVHR) conditions in mice, combining variations in the nature of the following: the F1 hosts (newborn or adult, normal or lethally irradiated), the injected parental T cells (mixed or selected subsets of Lyt2$^+$ or L3T4$^+$ cells), and the antigenic stimulus (semi-allogeneic or restricted to class I or II MHC loci).

The following conclusions were drawn: (*a*) Three gut alterations are always associated: (*i*) donor T cell infiltration, predominating in the crypt region; (*ii*) acceleration of the epithelium renewal; and (*iii*) increased epithelial Ia expression. (*b*) The initial event is T-cell infiltration, which results from stimulation within the Peyer patches followed by cyclic traffic, i.e., migration into the thoracic duct and then seeding to the whole gut mucosa. (*c*) Both Lyt2$^+$ and L3T4$^+$ cells can infiltrate the gut wall, the extent of the infiltration by a given subset depending upon (*i*) the capacity of the donor blasts to circulate in the thoracic duct (higher for L3T4$^+$) and then to home in the gut (much higher for Lyt2$^+$ blasts) and (*ii*) the nature of the alloantigenic stimulation that governs the extent of each donor subset proliferation. (*d*) Both donor T-cell subsets can induce gut epithelial damage, but for a comparable amount of infiltrating cells, L3T4$^+$ cells induce more lesions. (*e*) When the antigenic stimulation is restricted to class I or class I MHC loci, gut GVHR is much more easily elicited across class II MHC differences, which stimulate preferentially L3T4$^+$ donor cells. (*f*) The main mechanism of epithelial damage is not direct cytotoxicity, but more probably lymphokine(s) release.

Introduction

Graft-vs.-host reaction (GVHR)[1] occurs when foreign T lymphocytes are introduced into a host that, due to immunological incompetence or genetic reasons, is incapable of efficiently rejecting the grafted T cells. The alloantigens borne by the host cells are responsible for the activation of the donor T cells, which results in a chain of interactions between host and donor cells. The activated donor T cells are able to destroy or, conversely, to stimulate certain of the host cells (1–3) under conditions that are incompletely understood. One of the main target organs of this complex reaction is the gut, and in man diarrhea is among the earliest manifestations of acute GVHR.

Our present study explored the pathogenesis of intestinal lesions during GVHR in mice under various experimental conditions. Newborn or either lethally irradiated or nonirradiated adult mice were studied during the course of GVHR induced by the injection of donor T lymphocytes that varied in their degree of histoincompatibility (i.e., semi-allogeneic or differing only at class I or class II MHC loci) and also in their composition (i.e., total T lymph node [LN] lymphocytes or selected Lyt 2$^+$ or L3T4$^+$ subsets). Donor T cells isolated from the gut wall or obtained from the thoracic duct (TD) were studied for their surface phenotype and for their functional properties in the presence of host cells. The extent of gut epithelial alterations was determined in vivo, under various conditions of donor T-cell traffic, by a sensitive autoradiographic technique assessing the rapidity of the epithelial cell renewal. Our observations, reported here, led us to the conclusion that the basic situation responsible for intestinal tissue damage is the antigenic stimulation of donor T cells in the gut wall. This situation may be similar to that responsible for the lesions of various intestinal diseases in which T cells, activated in the Peyer's patches (PP) and seeding into the gut wall, are further stimulated by repeated contact with the antigen.

Methods

Animals and in vivo treatment. C3H (H_2k), DBA/2 (H_2d) mice were obtained from Centre National de la Recherche Scientifique, Orleans, France. Mice with recombinant haplotype, B10A (H_2a), B10T6R (H_2y_2) (6R), and AQR (H_2y_1) were kindly provided by M. Pla (Institut National de la Santé et de la Recherche Medicale [INSERM] U 93, Hôpital St. Louis, Paris, France). B10A and AQR have the same Ia specificities and differ at class I MHC K locus while 6R and AQR differ at class II (IA + IE) but not at class I MHC K locus (4). F_1 mice (C3H × DBA/2) (B10A × AQR) (6R × AQR) were raised in our animal house. Axenic mice (C3H) were a gift of R. Ducluzeau (Institut National de la Recherche Agronomique, Centre National de la Recherche Zoologique, Jouy en Josas, France) and were maintained in a sterile isolator.

GVHR were produced by injecting parental cells into F_1 mice. The injection of syngeneic lymphocytes into control mice was discontinued after preliminary experiments showed no difference between them and noninjected control mice (the data included the autoradiographic data mentioned below). (C3H × DBA/2)F_1 mice received C3H lymphocytes. We used a mixture of peripheric lymph node (PLN) and mesenteric lymph node (MLN) lymphocytes (unseparated or enriched for Lyt 2$^+$ and L3T4$^+$ subsets [see below]), but did not use spleen cells, to avoid injecting hematopoietic precursor cells. The recipients were either irradiated (900 rad) and nonirradiated adults or newborn F_1 mice. (*a*) In preliminary experiments, we determined the number of cells required to elicit in irradiated adult mice (as assessed by the spleen weight) the strongest GVHR without mortality 6 days after the injection. Semi-allogeneic mice received intravenously either 8×10^6 total LN, or 4×10^6

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1. *Abbreviations used in this paper:* GVHR, graft-vs.-host reaction; IE, intraepithelial; IEL, intraepithelial lymphocytes; IFN, interferon; LN, lymph nodes; LP, lamina propria; MAB, monoclonal antibody; MC, mast cells; MLN, mesenteric lymph nodes; PLN, peripheral lymph nodes; PP, Peyer's patches; and TD, thoracic duct.

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L3T4$^+$, or 12 × 10^6 Lyt 2$^+$ cells. Semicongeneic mice, with a difference across the class I MHC locus, received 20 × 10^6 total LN or L3T4$^+$ or 20 to 40 × 10^6 Lyt2$^+$ parental cells with class I MHC locus incompatibility, and either 10 × 10^6 total LN or 6 × 10^6 L3T4$^+$ or 20 × 10^6 Lyt 2$^+$ parental cells with class II MHC locus incompatibility (all preparations of parental cells contained comparable amounts, 20–30%, of B cells). With doses of parental cells higher than those indicated, mortality was frequently observed before the 6th day, except across class I or with Lyt2$^+$ cells across class II MHC locus incompatibility. (*b*) In all combinations used, newborn mice received 10–15 × 10^6 parental lymphocytes intraperitoneally during the first 48 h of life (lower amounts of parental cells were not tried and larger amounts found impractical). (*c*) Semiallogeneic nonirradiated adult F_1 mice received 100 × 10^6 LN cells intravenously in two equal doses (on days 0 and 7), a protocol derived from that of Rolink and Gleichmann (3). Viable cells (as judged by trypan blue exclusion) were injected intravenously at the rate of 1 × 10^6 cells/3 s.

TD cannulations were performed as previously described (5). Fetal gut grafts were placed subcutaneously at least 20 d before induction of the GVHR. Hematocrit measurement and direct Coomb's tests were performed as described (2).

Preparation of cell suspensions. Cell suspensions from lymphoid organs, gut intraepithelial (IE), and lamina propria (LP) cells were prepared in adult mice as previously described (6, 7). LP cells represent mucosal lymphohaematopoietic cells devoid of villous epithelium IE cells and are referred to as "gut lymphocytes." In newborn mice, the epithelium was not removed before the isolation of gut lymphocytes.

Separation of T-cell subsets. A combination of cytotoxicity and panning procedures was used. Elimination of Lyt 2$^+$ cells or L3T4$^+$ cells were performed by incubating lymphocytes with rat anti-Lyt 2 (14281) or L3T4 (GK 1.5) monoclonal antibodies (MAB) (kind gifts of F. Fitch, University of Chicago). An anti-rat kappa chain MAB (kind gift of H. Bazin, University of Louvain, Belgium) was added to lyse L3T4 cells because the GK 1.5 antibody is not cytotoxic. The cells were incubated in dishes coated with goat anti-mouse Ig; then the nonadherent cells were treated with complement (final dilution 1/10, Low-tox M rabbit complement, Cedarlane Laboratories, Hornby, Canada). The purity of the separated cells was verified by immunofluorescence after each separation. These populations contained very few (0.01–2%) residual lymphocytes of the eliminated subset, but ~20–30% of non–T cells, as in nontreated populations.

Immunofluorescence, histological and cytological procedures, thymidine (^{3}HTdR) labeling, and autoradiography. Cells were stained separately or in combination with a rhodamine-labeled rabbit anti-Thy 1 antibody (8) or with the following MAB: rat anti-Lyt 2 (AT 104), anti-L3T4 (described above), and anti-Thy (AT 83) (gift of F. Fitch). These MAB were revealed by a biotin rabbit anti-rat Ig (absorbed on mouse Ig) followed by fluoresceinated avidin. Cells bearing the H_2^d or the H_2^k antigens were stained by an anti-H_2 alloantisera (prepared in mice of one haplotype injected with spleen cells of the other [DBA/2 and C3H]); then, the cells were stained by fluorescein-labeled goat anti-mouse Ig (Nordic Immunological Laboratories, Tilburg, Netherlands) and rhodamine-labeled anti-Thy 1 antibody.

For fluorescence studies of tissues, blocks were frozen in isopentane fluid cooled in liquid nitrogen and cryostat tissue sections were fixed in acetone. Staining was performed with the antisera described above. For the detection of Ia-positive cells, a biotin anti-Ia k-d MAB (gift of M. Pierres, INSERM, Marseille Luminy, France) or an A.TH anti-A.T1 antiserum (gift of M. Pla) were used in (C3H × DBA)F_1 and in congeneic mice, respectively. IgA plasma cells and kidneys were stained as described (9, 10).

For histological and autoradiographic studies, tissues were fixed in Carnoy's fluid. Sections were prepared at various levels of the small and large bowel, but autoradiographs were always performed on sections of the duodenum. We measured the extent of labeling of the epithelial cells along the axis of the villi with an ocular micrometer. For detection of gut mast cells (MC), we processed tissues as previously described (7).

Labeling of dividing cells in vitro was carried out by 1-h incubation with 1 μCi/ml ^{3}HTdR and in vivo by a single injection of 1 μCi ^{3}HTdR/g body weight (5 C/mMol; Centre de l'energie atomique, Saclay, France). For autoradiography, Ilford K5 liquid emulsion (Essex, England) was used.

Cytotoxicity tests were performed on tumor target cells as described by Cerottini et al. (12) using: P 815 (H_2^d) and P 388 D1 (H_2^d), whose spontaneous bearing of Ia molecules was increased by a 48-h culture in the presence of 75 IU/ml of purified rat interferon (IFN) (as described [13]) and EL (H_2^b).

Cell cultures and studies of supernatant activities. For the detection of interleukin release, lymphocytes were cultured in Dulbecco's supplemented medium (Gibco Europe Ltd., Uxbridge, Middlesex, England) with 10% fetal calf serum, using 1 × 10^6 cells/ml in the presence of 2.5 × 10^6 irradiated (2,000 rad) spleen cells/ml of mice of host F_1 phenotype, depleted in Thy 1$^+$ cells by treatment with MAB and complement. Interleukin assays were performed as described (7) with the use of an IL3-dependent cell line (a gift of T. M. Dexter, Manchester, England) and of the CTLL IL2–dependent line (a gift of S. Gillis, Hanover, New Hampshire). IFN was titrated by inhibition of the cytopathic effect of vesicular stomatitis virus in a mouse transformed fibroblastic line (L 929). The results were expressed in International units by the use of an IFN standard (T 102 12.8 × 10^6 IU ml, kindly provided by I. Gresser, Institut Gustave Roussy, Paris).

Results

Description of the gut alterations during GVHR

The results observed in the three models used, namely injection of F1 T cells into lethally irradiated or normal adult or newborn parental hosts, are shown on Tables I, II, and III, respectively. The most detailed results were obtained with lethally irradiated hosts, in which T-cell traffic in the TD and T-cell subsets composition of LN were also studied (Table I); indeed, in irradiated hosts virtually all dividing T cells recovered were of donor origin, allowing an easier study of the functional properties of these cells, as described below. In these three models of GVHR, the constant gut alterations, i.e., the landmarks of intestinal disease, were (*a*) the infiltration of the gut mucosa by donor T cells and (*b*) the epithelial damage, whose most sensitive index was the acceleration of the epithelial renewal.

T-cell infiltration of the gut

T-cell infiltration was studied both on tissue sections by immunofluorescent staining, and using cell suspensions isolated either from the villous epithelium or from the LP and crypt epithelium (see Methods).

In control mice, noninjected with parental T cells, the presence, nature, and distribution of gut lymphocytes depended on the condition of the animals: newborn mice were devoid of gut lymphocytes (6); in adult mice, lymphocytes were scattered among the villi and were very rare in the crypts, L3T4$^+$ cells predominating in the LP and Lyt 2$^+$ cells (bearing or not the Thy 1 antigen) (14–16) within the epithelium; in adult irradiated mice, only few nonproliferating cells persisted in the LP, but some Lyt 2$^+$ cells, mostly Thy 1$^-$ and dividing, are found within the epithelium (as judged on tissue sections and on isolated cells incubated with ^{3}H TdR).

In the three models of GVHR studied, the T-cell infiltration of the gut had similar features in its localization, severity, and composition. It predominated in the crypt area, the LP, and the epithelium of the crypts, i.e., in a localization in which lymphocytes were rare in normal mice; this was observed along the entire length of the gut. The infiltration was massive when observed at day 6 for irradiated mice and around day 12 for unirradiated adult or newborn mice, in GVHR elicited by total parental lymphocytes in semi-allogeneic mice, with the number of cells used (Tables I–III, Figs. 1 and 3). The intensity of the infiltration can be best assessed in lethally irradiated animals: on

*Table I. T Cell Subsets and Gut Lesions in GVHR in F_1 Adult Lethally Irradiated Mice**

		Isolated lymphocytes							
		Total lymphocytes			Rapidly dividing T blasts‡		Gut lesions		
	Lymphocytes used to elicit GVHR	Recovered from	% Lyt2+	% L3T4+	% Labeled cells among total cells	% Lyt 2	% L3T4	T-cell infiltration	Index of epithelial renewal‖	Ia on crypts cells
Semi-allogeneic GVHR§	Total 8 × 10^6	Gut¶	81±3.3	16±5	19±6	83±1.4	16±1	+++	2–3	+++
		TD	32.5±6.0	67±5.8	11±1.9	60±5.3	40±4.3			
		MLN	85	14	12	93	7			
		PLN	77.5	22.5	16	88	12			
	Lyt2+ 12 × 10^6	Gut	98±0.9	ND	15.5±4.9	99.9 (0)	ND	+++	2–3	+++
		TD	96.8±0.6	ND	21.5±0.5	99.4 (0)	ND			
	L3T4+ 4 × 10^6	Gut	ND	46±9**	18±8.4	ND	99±0.6	++	2–3	+++
		TD	ND	98.7±0.1	5.5	ND	99±1.2			
Semi-congeneic GVHR across cl.I MHC locus	Total 20 × 10^6	Gut¶	64	18.5	8.2	83	14	++	2	+++
		TD	32.5±2.1	71±5.6	23±3.1	52.5±10	46.5±10			
		MLN	82	13	17.5	93.6	5.6			
		PLN	76	15	12	91	9.4			
	Lyt2+ 20 × 10^6	Gut	ND	ND	ND	ND	ND	+	1.4	++
	Lyt2+ 40 × 10^6	Gut	ND	ND	ND	ND	ND	++	1.9	++
	L3T4+ 20 × 10^6	Gut	ND	ND	ND	ND	ND	+	1.7	+++
		TD	3±0.1	96.5±0.7	6.5±0.7	5±2.6	94±1.4			
Semi-congeneic GVHR across cl.II MHC locus	Total 10 × 10^6	Gut¶	42‡‡	50‡‡	10	49	48	+++	2–3	+++
		TD	15.7±0.3	87±3.2	16±1.4	20±2.8	79.5±0.7			
		MLN	26.5	68	12	40	59.5			
		PLN	27	68	12	32	68			
	Lyt2+ 20 × 10^6	Gut	84±5	10±2.5	12.5	91	9	++	1.3	+++
		TD	80±3.2	20±3.6	23±8.7	89.5±1.8	11.5±1.1			
	L3T4+ 6 × 10^6	Gut	ND	ND	ND	ND	ND	+++	2–3	+++

* For TD and gut the results were the average of two to five experiments. TD was cannulated on the 4th or 5th day, and all mice killed on the 6th day. ‡ ^{3}HTdR labeled in 1-h in vitro incubation. (The residual lymphocytes from host origin were nondividing cells.) ‖ Ratio of the extent of ^{3}HTdR labeling along the villi (measured with a micrometer) in GVHR/control conditions. § In these conditions of GVHR, the origin of the lymphocytes was studied with an anti-H_2 antiserum; 94–99% of the cells are T lymphocytes of donor origin, except after injection of donor L3T4 cells (see **). ¶ Gut lymphocytes were obtained from the LP and the crypt epithelial cells after removing of the villous epithelium (see Methods). Isolated PP lymphocytes were often contaminated by epithelial cells and by lymphocytes of the adjacent gut because of their small size in GVHR; thus they were not accurately studied. On gut sections, however, the number of Lyt 2+ and L3T4+ PP lymphocytes correlated with that of PLN and MLN lymphocytes. For GVHR across class I and class II loci, complete exploration of the isolated gut lymphocytes, as shown on the table, was performed only once, but was closely comparable to results obtained by immunofluorescence of tissue sections. ** In this model, a significant amount of host cells always persisted among the recovered cells. Besides, the recovery was smaller: 1.4×10^6 SD 0.7 per mouse in contrast with the recovery obtained after total lymphocyte injection: 3.7×10^6 SD 1.2 per mouse and after Lyt 2+ subset injection 3.9×10^6 SD 0.7. Donor T cells were nearly all L3T4+ (as shown by the identical number of lymphocytes stained with anti-Lyt 2 antibody and with a mixture of this antibody and anti-H_2 antibody). ‡‡ % Granulated lymphocytes on May Grunwald Giemsa stained smears. PLN 0.4%, MLN 2%. Gut lymphocytes (after Vernier localization of fluorescent Lyt 2+ or L3T4+ cells): Lyt 2+ lymphocytes 60%, L3T4+ 11%.

the 6th day of the GVHR, more lymphocytes (3.7 × 10^6 ±1.2 (SD) per mouse) were recovered from the wall of the small bowel than from the pooled lymphoid organs, spleen, and all detectable LNs. Finally, the T-cell infiltration consists of about 4/5 of Lyt2+ and 1/5 L3T4+ cells, as detected on isolated cells obtained from the LP and the crypt epithelium (Tables I, II [see ‖‖], and III [see ‖‖]), with a high proportion of dividing cells. 25–35% of these cells contain the small but characteristic granules that have been previously described (6). Using an anti-H_2 alloantiserum, it has been verified that almost all these cells were of donor origin (Tables I [see §], II, and III), except in unirradiated adult mice, where the proportion of donor and host T cells was somewhat more variable (Table II), as was expected, since these animals have a high content of gut T lymphocytes before the induction of GVHR. The donor T cells found in the LN of irradiated hosts were also almost all Lyt2+, but this was not true of the T cells circulating in the TD (Table I): the significance of this observation will be discussed below.

The following questions were then asked:

(*a*) Since both Lyt2+ and L3T4+ donor T cells are found in the gut lesions (although with Lyt2+ cells vastly predominant), can purified donor T cells of each of these subsets induce a GVHR in semi-allogeneic hosts?

In adult irradiated hosts, both cell types elicited a gut GVHR,

*Table II. Gut Lesions and Their Evolution during the Course of GVHR in Semi-allogeneic Nonirradiated Adult Mice Injected with Parental T Lymphocytes**

			Gut				
	Day of killing‡	Hematocrit	% Donor lymphocytes‖	Index of epithelial renewal§	Ia on crypt epithelial cells	MC¶	IgA plasma cells**
Control mice		42–48			0	0–1	22–42
GVHR elicited by	11	42	26.5‡‡	ND	++	10–50	20
total lymphocytes	13	39	74–93‖‖	1.5	++	2.5	5
	19	28	46	1.7	+	0.4	2
	20–25	Death					
GVHR elicited by	12	40	52	1.5	++	2	16
$Lyt2^+$ subset§§	19	32	33	1.7	++	7	8
	22–60	42	1.5	1	0	0	25
GVHR elicited by	9–13	44	1.5	1.3	0	8.5	29
$L3T4^+$ subset¶¶	75	42	0	1	0	0	26

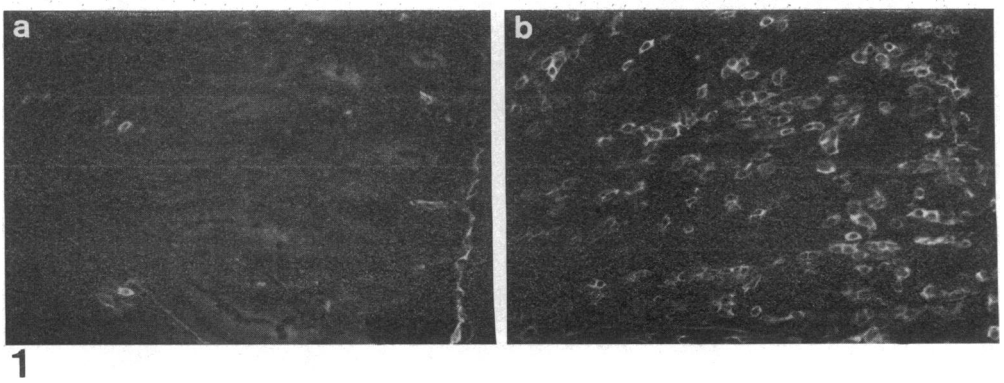
* 10^8 lymphocytes injected i.v. in two equal dosages on days 0 and 7. ‡ For each day studied, average of at least two experiments. ‖ Among lymphocytes isolated from LP and crypt epithelial cells. § As in Table I. ¶ Per 10 villous unit as described (7). ** Per villous unit. ‡‡ At this time, there was a strong stimulation of donor T cells (15% labeled after 1-h ^{3}HTdR incubation). ‖‖ Lyt 2^+ cells: 86%; $L3T4^+$ cells: 11%; ^{3}HTdR labeled: 8%. Both populations were dividing. §§ Half of these mice died around day 20. ¶¶ All the mice survived. As early as day 12, their kidneys usually contained immunoglobulin deposits and direct Coomb's test may be positive as described (3, 10).

but different cells numbers had to be used: in order not to cause death before the 6th day, it was necessary to inject threefold fewer L3T4 than $Lyt2^+$ cells (and twofold fewer L3T4 than total T cells) (Table I); this fact suggests that $L3T4^+$ cells proliferate earlier than $Lyt2^+$ cells after injection into the irradiated host. Furthermore, to get the same T-cell infiltration and epithelial lesions as total T cells get, more $Lyt2^+$ cells than total T cells (12 vs. 8×10^6) were necessary; that this last finding does not simply reflect a small degree of contamination of $Lyt2^+$ cells by $L3T4^+$ cells was indicated by the fact that virtually all cells recovered from the gut mucosa and the TD in GVHR induced by selected $Lyt2^+$ cells were indeed $Lyt2^+$ (Table I). Injection of 4×10^6 parental $L3T4^+$ cells was sufficient to induce gut lesions; with the same number of $Lyt2^+$ cells, only minimal lesions were observed (data not shown); however, while the epithelial lesions (discussed below) were similar to those of mice injected with 8

*Table III. Gut Lesions in F_1 Newborn Mice with GVHR Induced by Injection of Parental Lymphocytes**

		Gut					
	Hematocrit	% Donor lymphocytes‡	T lymphocytes infiltrate‖	Index epithelial renewal§	Ia on epithelial cells	MC¶	IEL ratio $Lyt2^+/Thy1^+$**
Control mice							
Day 10–14	30		0		0	0	—‡‡
Semiallogeneic mice: GVHR:							
Elicited by total lymphocytes	19	97‖‖	+++	3–6	+++	4	1§§
Elicited by $Lyt2^+$ subset	21	55	+++	3–6	+++	2	1–2§§
Elicited by $L3T4^+$ subset¶¶	23	5	+	1.5–4	++	12	2§§
Semi-congeneic mice: GVHR:							
Across cl.I MHC locus							
Elicited by total or $L3T4^+$ lymphocytes	30	ND	0	1	0	0	0
Across cl.II MHC locus							
Elicited by total lymphocytes	28	ND	+	3–4	+++	12	2
Elicited by $Lyt2^+$ subset	25	ND	+	3–4	+++	2	2
Elicited by $L3T4^+$ subset¶¶	25	ND	+	7–9	+++	23	2

* All mice received i.p. 10×10^6 lymphocytes and were killed between day 10 and 14. The results are the average of three to twelve experiments. ‡ Among the lymphocytes isolated from the whole gut (LP + epithelium) of four mice. ‖ Judged by staining of tissue sections with anti-Thy 1. § As in Table I. ¶ As in Table II. ** Lyt 2^+ and Thy 1^+ cells numbered by fields on tissue sections in the epithelium. In normal adult mice this ratio was around 2. ‡‡ These cells were absent in normal mice before days 30 to 40. ‖‖ Lyt 2^+, 83%. $L3T4^+$, 14%. §§ The recovery of Lyt 2^+ Thy 1^- lymphocytes obtained with isolated lymphocytes showed a good correlation with the observation made on tissue sections. When the ratio was 1, no Lyt 2^+ Thy 1^- cells were found among isolated lymphocytes. When the ratio was 2, numerous Lyt 2^+ Thy 1^- were found as in adult mice. ¶¶ Immunoglobulin deposits in the kidneys of these mice were already conspicuous on day 11 and were still observed when the mice have no more detectable donor T cells and intestinal injury by all criteria studied (days 30 and 120).



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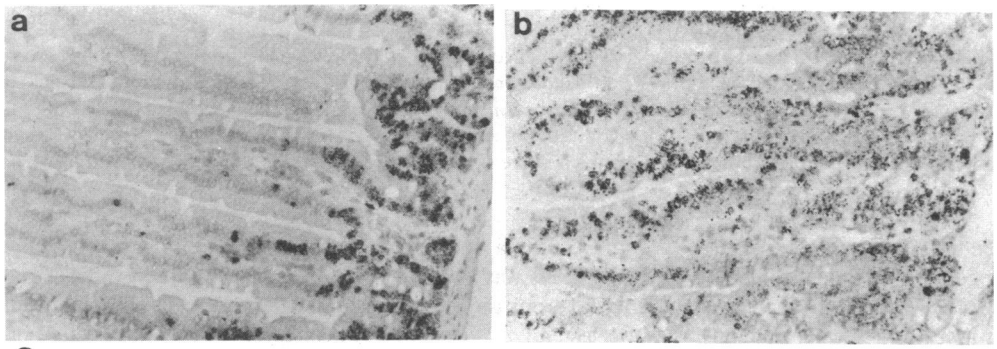

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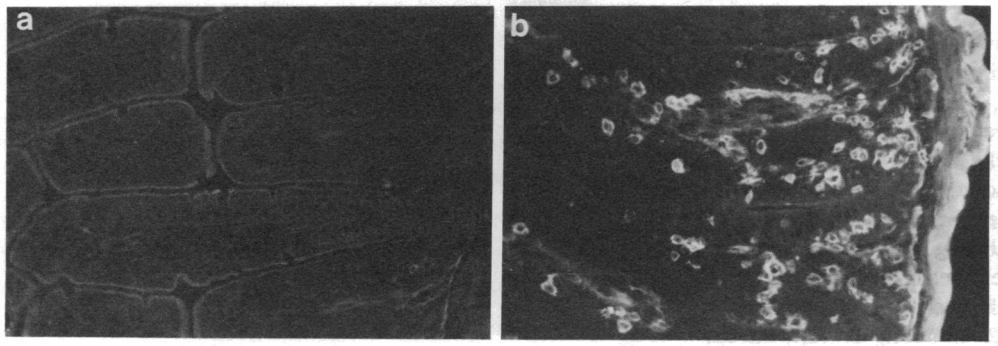
Figures 1 and 2. Duodenum sections from lethally irradiated (C3H × DBA)F_1 adult mice killed on day 6. (*a*) Uninjected control. (*b*) GVHR (elicited by total parental lymphocytes). Fig. 1 immunofluorescence with anti-Thy 1 antiserum. Fig. 2 autoradiographies 26 h after in vivo injection of ^{3}HTdR. 2-mo exposure, methyl-green-pyronin staining. The index of epithelial renewal is 3.

× 10^6 total T cells, the T-cell infiltration was less severe (Table I [see **]). Since practically all the gut mucosa and TD blasts were indeed L3T4$^+$ in this type of GVHR, there was again no indication that a significant contamination by the other T-cell subsets might play a role in the gut lesions. In nonirradiated adult and newborn mice, injection of Lyt2$^+$ parental cells also led, on day 12, to marked gut lesions and massive T-cell infiltration (Tables II and III). In contrast, with L3T4$^+$ cells, there was almost no detectable change in the adult gut (Table II), while in the newborn mice a minimal T-cell infiltration was associated with quite conspicuous epithelial changes (Table III). In conclusion, it appears that both donor T-cell subsets can independently create gut damage, but that activated Lyt2$^+$ cells have a greater tendency to home into the gut, while L3T4$^+$ cells in contrast have a greater capacity to create gut damage, since a smaller L3T4$^+$ cell infiltration is associated with significant epithelial damage.

(*b*) What are the antigenic stimuli responsible for the gut GVHR? Can gut GVHR be observed in hosts differing from the donor T cells only in class II or class I MHC antigens, and, in this case, what is the involvement of each of the two T-cell subpopulations?

In irradiated mice, gut GVHR could be observed across either class I or class II MHC antigenic differences, but to elicit lesions of comparable intensity, fewer parental T cells (half or less) were required across class II than across class I differences (Table I). It has to be noted that, with the congeneic mice used, the results were different when the irradiated hosts were from the other parental lineage rather than F1 mice: in this case, a very small number of T cells (0.2–0.5 × 10^6) elicited strong GVHR across class II differences (data not shown), agreeing with the observations of Piguet (17). Marked gut lesions were observed in GVHR elicited across class II antigeneic differences (A + E) by injection of unselected 6R donor T cells into (6R × AQR) F1 hosts. However, the composition of the lymphocyte infiltration is different from that observed in GVHR elicited across complete allogeneic disparity, since it consisted of equal proportions of L3T4$^+$ and Lyt2$^+$ cells; this suggests that this more limited antigenic disparity preferentially stimulates L3T4$^+$ cells, as was also seen in the LN, where most cells are L3T4 (Table I). One third of the gut lymphocytes are granulated (Fig. 6), the vast majority of which were Lyt2$^+$ (see table I FN 7), while lymphocytes proliferating in LN are devoid of granules (Fig. 7). When selected donor T-cell subsets were used to elicit this form of GVHR across class II antigens, both subsets lead to T-cell infiltration and gut lesions, but fewer L3T4$^+$ than Lyt2$^+$ cells (about threefold) are required (Table I; note, however, that from the composition of the gut T cells recovered after Lyt2$^+$ cells injection, a contribution of contaminating L3T4$^+$ cells among Lyt2$^+$ cells cannot be excluded). In GVHR elicited across class I antigenic difference (K) by injection of unselected B10A T lymphocytes into (B10A × AQR) F1 irradiated mice, gut lesions were also observed, but the T-cell infiltrate consists predominantly of Lyt2$^+$ cells (Table I), in contrast to class II GVHR. When selected L3T4$^+$ cells were injected into these F1 recipients, there was moderate gut damage and the T-cell infiltration was weaker (Table I), often focal rather than diffuse (immunofluorescence on tissue sections confirmed its L3T4$^+$ nature). Selected Lyt2$^+$ cells were also capable of inducing gut lesions, but very large number of donor cells were required (Table I). Similar experiments were performed in newborn mice (Table III): with the donor cell amount used, no GVHR gut lesions were seen across class I difference, while across class II differences injection of either total T lymphocytes or selected subsets lead to a mod-



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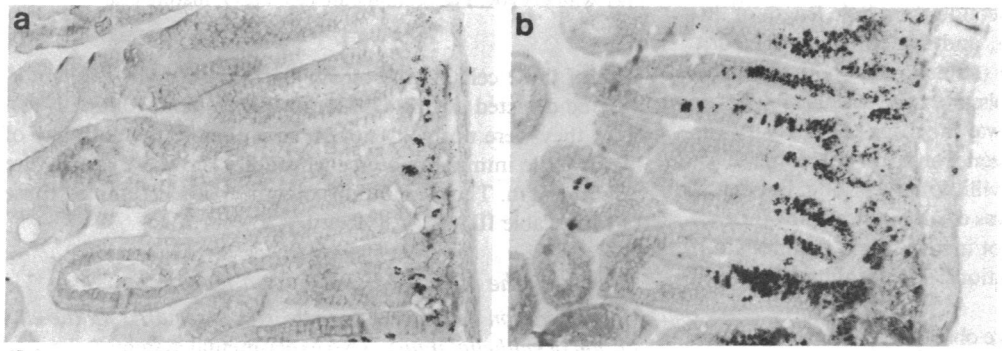

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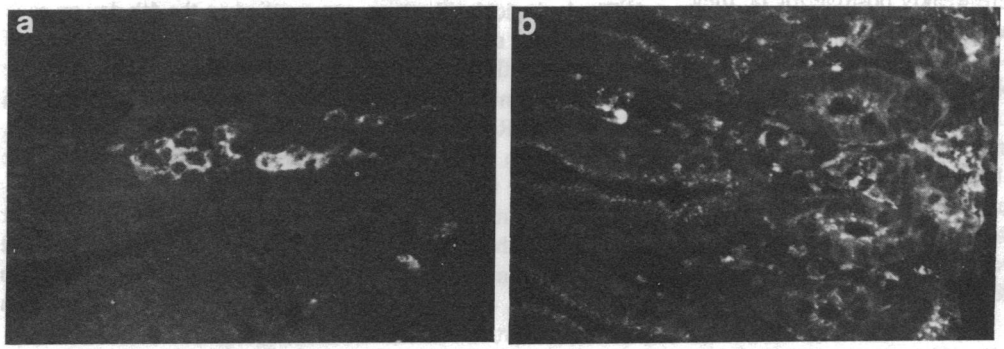

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Figures 3, 4, and 5. Duodenum sections from 12-d-old (C3H × DBA)F_1 mice. (*a*) Control. (*b*) GVHR (elicited by total parental lymphocytes). Fig. 3 immunofluorescence with anti-Thy 1 antiserum. Fig. 4 autoradiographies after ^{3}HTdR injection as in Fig. 2. The index of epithelial renewal is 5. Fig. 5 immunofluorescence with anti-Ia antibody. (*a*) Ia^+ cells are abundant in the LP of the villi and the epithelium is not stained. (*b*) The epithelium of the crypts and of the villi is strongly positive; in contrast, there are fewer Ia^+ cells in the LP.

erate gut T-cell infiltration, but associated with marked intestinal lesions. In conclusion, it appears that class II MHC differences represent a definitely stronger antigenic stimulation than class I MHC difference in the elicitation of gut GVHR, and that this stimulation acts preferentially on $L3T4^+$ cells, in contrast to what is observed across class I MHC or full allogeneic differences.

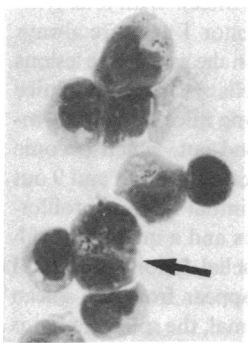


Figure 6. Cells isolated from the gut (LP + crypts) of (6R × AQR)F_1 mice injected with 6R total lymphocytes. Three blasts (one in mitosis: arrow) contain cytoplasmic granules.

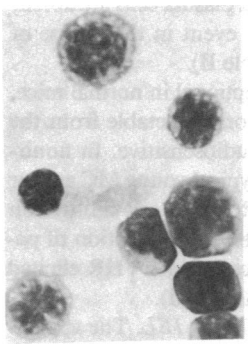


Figure 7. Cells isolated from the PLN of the same mice. Blasts do not contain cytoplasmic granules.

Gut epithelial cell alterations

Acceleration of the epithelium renewal. Renewal of the epithelium can be evaluated with precision, using autoradiographic analysis after in vivo injection of ^{3}H TdR. 3 h after ^{3}H TdR injection, the only labeled epithelial cells were within the crypts, which were the area of proliferation of the villous epithelial cells; afterwards, labeled epithelial cells reached the top of the villi within 2 or 4 d (18). Thus, the speed of epithelial renewal may be assessed by the extent of the villi labeling 26 h after ^{3}H TdR injection. The index of increase in epithelial renewal, comparing GVHR to control conditions, is the most precise and sensitive way of detecting intestinal GVHR. In strong GVHR, the epithelial lesions are histologically conspicuous, with hyperplasia and sometimes dilatation and necrosis of the crypts on one hand, enlargment and shortening of the villi on the other. In such conditions, 26 h after ^{3}H TdR injection the whole length of the persistent villi is labeled in adult, and sometimes in newborn mice, while only the lower third (adult) or the bottom of the villi (newborn) is labeled in controls: thus, the calculated indexes of acceleration of epithelial renewal in strong GVHR vary between 2 and 3 in adults and between 3 and 9 in newborns (Figs. 2 and 4). In milder GVHR, the villi remained long, but acceleration of the epithelial renewal was clearly indicated by indexes around 1.5. When the index is not increased, no manifestation of gut GVHR (histological alterations, T-cell infiltration, or Ia expression) was seen.

The extent of epithelial damage observed in the various forms of GVHR studied, evaluated according to this index, is shown on Tables I, II, and III.

Appearance or increase of Ia molecules. In control adult mice, the epithelial cells of the villi were weakly positive for Ia; they are negative in newborn mice until around day 30 (which is the time of T-cell appearance). In all mice showing GVHR, Ia expression is increased, detectable in the crypts and sometimes on the whole length of the villi (Fig. 5 *b*), as indicated by the subjective scoring system used in Tables I–III.

Finally, the possible role of bacterial contamination of the gut in the genesis of these lesions was explored in axenic mice kept under sterile conditions. This showed that the T-cell infiltrate and the epithelial lesions were identical to those of similar mice kept in conventional conditions.

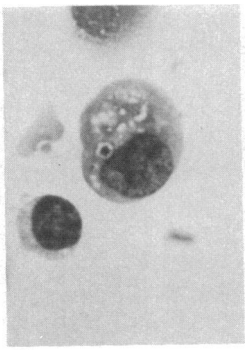
Effect of GVHR on gut host cells of haematopoietic origin

Decrease in LP Ia$^+$ cells, probably macrophages or dendritic cells. These cells, which were numerous in the LP since time of birth (Fig. 5 *a*), became much less conspicuous in all forms of strong GVHR (Fig. 5 *b*). It is important to point out that they are radioresistant.

Decrease in IgA plasma cells. This could only be clearly observed in adult, nonirradiated mice (gut plasma cells are absent in newborns) and was a relatively early event in the course of GVHR, which parallels its severity (Table II).

Appearance of MC. Gut MC are exceptional in normal mice, in spite of the presence of local precursors detectable from the time of birth (7); these precursors are radiosensitive. In nonirradiated mice, either adult or newborn, typical young MC appear and mature in the gut at the time of donor T-cell infiltration (Figs. 8 and 9). Their number was greater after injection of parental L3T4 cells in semi-allogeneic mice, or in GVHR elicited across class II MHC antigens (Tables II and III).

Appearance of Lyt 2$^+$, Thy 1$^+$, or Thy 1$^-$ IEL. The appearance of these cells, numerous in normal mice (14–16), could only be detected during GVHR in the newborn (days 10–13), since they were normally not observed before the 30th day of life. Double immunofluorescence studies showed that they are of host origin. Their conditions of appearance resembled those of MC (Table III).



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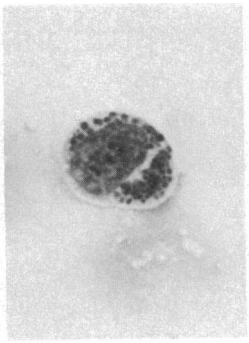

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Figures 8 and 9. MC isolated from the gut of nonirradiated adult F_1 mice with GVHR. Fig. 8, immature cell. Fig. 9, mature cell.

Studies on the mechanisms of the gut lesions

Gut infiltration by donor T lymphocytes is required for the elicitation of epithelial injury. Two experimental systems were used to explore the relationship between donor T-cell infiltration and epithelial lesions.

GVHR were elicited in lethally irradiated mice, and [^{32}P]polyvinylchloride strips were applied on the 4th day on each PP. We have shown previously that this selective irradiation of PP prevents the accumulation of donor T lymphocytes in the gut wall but not their proliferation in lymphoid organs (6). On day 5, ^{3}HTdR was injected and the mice were killed on day 6. Compared to GVHR control mice without PP irradiation, the experimental mice showed not only very limited T-cell infiltration in the gut (6), but also a normal epithelial renewal, similar to that of lethally irradiated mice not undergoing GVHR: the crypts remained low, and only their bottom was labeled with ^{3}HTdR (Fig. 10). Thus, there is a good correlation between T-cell infiltration and epithelial alterations.

GVHR were also elicited with total or selected T-cell populations in nonirradiated semi-allogeneic adult and newborn mice, and the persistence and localization of the donor T cells (detected by double immunofluorescence staining with anti-Thy 1 and anti-H_2 antibodies) was followed in relation to the presence and evolution of intestinal lesions. The course of the disease varies with the nature of the T lymphocytes used to elicit the GVHR (Table II, not shown for newborn mice). With total lymphocytes, death occurs before day 25; donor T cells are always present in the spleen (often necrotic) and in the gut, whose lesions remain marked. With selected Lyt 2$^+$ cells, ~50% of the mice survive after day 20, and in these surviving mice, donor T lymphocytes disappear from both spleen and gut, which become normal (Table II). With selected L3T4$^+$ cells, all adult and 9 out of 10 newborn mice survived. After an initial marked proliferation of donor T cell in lymphoid organs and a moderate infiltration of the gut associated with epithelial lesions (Tables II and III), the donor T lymphocytes disappear from the spleen and the gut. While the gut returns to normal, the spleen remains

enlarged with large germinal centers, immunoglobulin deposits are conspicuous in glomeruli (and sometimes tubular basement membranes as well), and the Coomb's test is positive with erythrocytes, which agrees with the observations of Rolink and coworkers (3, 10). In conclusion, all these experiments show a good correlation between donor T-cell persistence, gut infiltration, and epithelial lesions.

During GVHR, the two subpopulations of T blasts are different in their gut-homing and circulating properties. We have previously shown that the donor T cells infiltrating the gut during GVHR are mostly the progeny of blasts (proliferating cells incorporating ^{3}H TdR) originating by allogeneic stimulation in the PP and disseminating all along the gut mucosa through migration to the MLN, TD, and blood, due to their special gut-homing ability (6). As shown above, in all forms of semi-allogeneic GVHR elicited by the injection of total LN lymphocytes (containing about equal amounts of Lyt2$^+$ and L3T4$^+$ cells), >80% of the donor T cells infiltrating the gut are Lyt2$^+$. This, however, does not reflect the repartition of the donor blasts circulating in the TD, since 40% are L3T4$^+$ in irradiated adults (Table I). On the other hand, in the same mice, the composition of the TD blasts also differ from that of the blasts proliferating in the LN and the PP, where it is again >80% Lyt2$^+$ (Table I [see ¶]). This is compatible with the possibility that Lyt2$^+$ TD blasts have a greater gut-homing tendency than L3T4$^+$ TD blasts, but that Lyt2$^+$ blasts found in the LN and the PP have themselves less tendency to circulate in the TD than LN L3T4$^+$ blasts. To explore directly the gut-homing properties of the subpopulations of TD blasts, GVHR were elicited with selected subpopulations, and the almost pure populations of Lyt 2$^+$ or L3T4$^+$ blasts obtained by TD cannulation of these mice (Table I) were labeled with ^{3}H TdR and used in transfer experiments into normal syngeneic mice, to explore their gut-homing tendency appraised by the ratio of labeled cells found in the gut and in the spleen on radioautographs (done as previously described [6, 9]). This experiment showed that Lyt 2$^+$ TD blasts have a markedly higher tendency to home into the gut than L3T4 TD blasts (ratio of migration in the gut vs. the spleen: 0.67 for Lyt 2$^+$ blasts, 0.13 for L3T4$^+$ blasts; no lung trapping of either blast variety was observed). Furthermore, among Lyt 2$^+$ TD blasts homing into the gut, 32.5% were located within the epithelium, compared to only 8% for L3T4$^+$ TD blasts. This difference in gut-homing tendency explains why the proportion of Lyt2$^+$ cells among the T cells infiltrating the gut mucosa was always higher than among TD blasts from which they arose in GVHR elicited either in semi-allogeneic conditions or restricted to class I or II MHC antigeneic differences (Table I). In all these conditions, too, the TD blasts always contained more L3T4$^+$ cells than the LN (Table I), which result agrees with the suggestion that L3T4 blasts have a higher capacity to circulate. This was illustrated by the observations made in GVHR across class II MHC locus, in which, as already mentioned, the L3T4$^+$ cells were preferentially stimulated: in this situation, the TD blasts were almost all L3T4$^+$ cells, while the gut T cell infiltration was made of both subsets in equal proportions (Table I), in contrast to what is observed in GVHR is semi-allogeneic conditions or across class I MHC locus. In conclusion, these observations provide strong evidence that Lyt2$^+$ blasts have less tendency to circulate in the TD than L3T4$^+$ blasts, but, in contrast, a higher tendency to home in in the gut when they have reached the TD.

Gut epithelial injury is observed even when donor T lymphocytes eliciting the GVHR are syngeneic to the epithelium. We have previously observed that in lethally irradiated F_1 mice bearing fetal gut grafts from both parents, the parental T lymphocytes eliciting the GVHR invade the grafts of either origin equally, i.e., syngeneic or fully allogeneic to the lymphocytes (6). Further experiments of this type showed that the acceleration of epithelium renewal (Fig. 11) and the induction of Ia molecules are identical in both grafts.

Donor Lyt 2$^+$ lymphocytes recovered from GVHR gut mucosa are specifically cytotoxic against host cells. T lymphocytes that were collected from the gut of mice undergoing semi-allogeneic GVHR displayed specific cytotoxicity for cells of the host MHC. This cytotoxicity was abolished in the presence of anti-Lyt 2 antibody, and was also found in GVHR elicited by selected Lyt 2$^+$, but not in GVHR elicited by L3T4 cells, even when the target cells used bore the host class II antigens (Table IV). It should be noted that GVHR TD lymphocytes do not show cytotoxic activity unless they have been treated previously with collagenase (in the conditions used for collecting gut lymphocytes), which may be related to the adsorbtion of host alloantigens on their membranes and their removal by proteolytic treatment (20).

Donor T lymphocytes recovered from GVHR gut mucosa release interleukines in vitro after specific allogeneic stimulation. The release in culture of IL3, IL2, and IFN by specifically stimulated donor gut T lymphocytes is shown on Table V. IL2 release was weak, but IL3 and IFN release was high. From GVHR elicited by selected donor Lyt 2$^+$ or L3T4$^+$ cells, it appears that L3T4$^+$ cells are more efficient in lymphokine release.

Discussion

In the three types of F1 hosts used (i.e., lethally irradiated, normal adult, or newborn mice), GVHR were induced by the injection

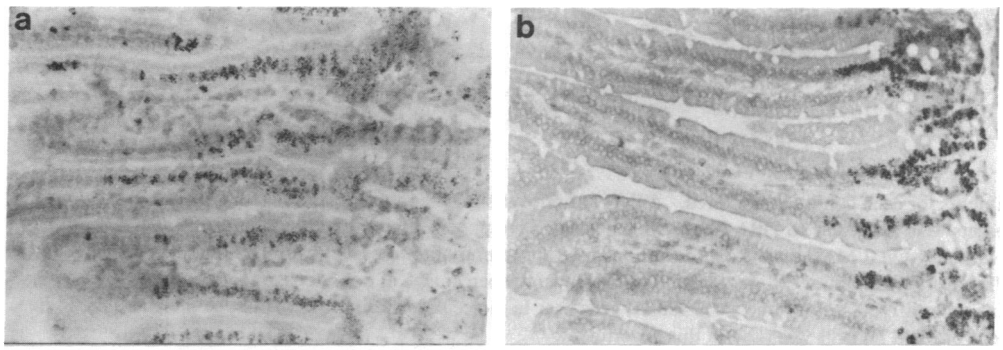

Figure 10. Duodenum sections from lethally irradiated (C3H × DBA)F_1 mice with GVHR elicited by total lymphocytes, killed at day 6 and 26 h after ^{3}HTdR injection. Strips of PVC without (*a*, control) or with ^{32}P (*b*) were glued on PP at day 4. Compare Fig. 10 *b* with 2 *a*.

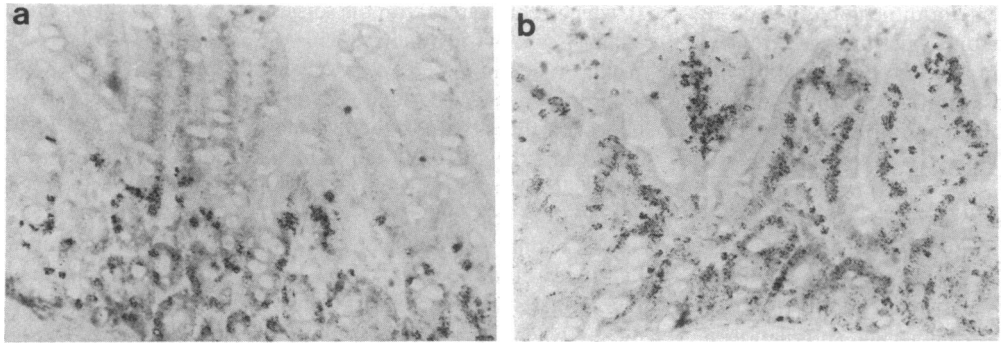

Figure 11. C3H fetal gut grafts borne by (C3H × DBA)F_1 mice. Mice were lethally irradiated 6 d before being killed. Autoradiographies after ^{3}H TdR injection as in Fig. 2. (*a*) Control mice: the crypts are labeled; (*b*) GVHR elicited by C3H lymphocytes syngeneic to the graft: the labeling extends the whole length of the villi.

of parental T cells of various compositions (mixture of Lyt2$^+$ and L3T4$^+$ cells in about equal amounts, or selected subpopulations). Furthermore, graft combinations were used in which the parental cells differed from the host at both major and minor histocompatibility loci, or only at class I (K) or class II (A + E) MHC loci. Although resulting in a complex variety of experimental conditions, this approach demonstrates a basic pattern of gut lesions in GVHR, and explains the involvement in these lesions of each subpopulations of parental T lymphocytes with respect to their functional properties and to the nature of the antigenic stimulation involved.

The three basic alterations found in the gut during GVHR were the following: (*a*) an infiltration of donor T lymphocytes predominant in the region of the intestinal crypts (both in the lamina propria and within the epithelium), a location where T cells are rarely found in normal mice; (*b*) an acceleration of the epithelial cell renewal, whose most sensitive index was the extent of labeling of the epithelial cells along the villi axis, as judged on radioautographs of gut sections obtained one day after a pulse of ^{3}H TdR (Figs. 2 and 4); and (*c*) an increase in Ia expression on the epithelial cell membranes, predominant within the crypts. These three alterations were always found concomitantly; in their absence, there was no other detectable intestinal alterations, even when there were marked GVHR lesions elsewhere. When these lesions were severe, T-lymphocyte infiltration can extend along the entire villi; the crypt epithelium shows an increased mitotic activity that was progressively accompanied by villous atrophy (21); crypt cell degeneration with crypt dilatation and necrosis, such as described in patients suffering from acute GVHR (22, 23), could be observed; there is strong Ia expression on the whole gut epithelium (24). When these alterations were very mild, they were more easily detected in GVHR of the newborn, since in normal newborn mice there was no gut T cells, a low epithelium renewal, and no Ia expression on the epithelium. The intestinal bacterial flora does not play an essential role in the development of these lesions, since strong lesions are observed in mice with GVHR maintained under axenic conditions.

That the donor T-cell infiltration is the initial event in these associated lesions was shown by two types of observation. First, selective irradiation of the PP, which has previously been shown (6) to selectively prevent the donor T-cell infiltration in the gut mucosa (because these cells arise by allogeneic stimulation in the PP and undergo cyclic traffic, migrating into the TD and then seeding to the whole length of the gut), also completely

*Table IV. Cytotoxicity of Gut T Lymphocytes from Mice with GVHR**

Lymphocytes used for eliciting GVHR‡		% Donor T cells among gut lymphocytes	Specific targets	^{51}Cr release from targets, Ratio lymphocytes/targets 3.6	11	33	100
Total	1	93	P815	8.5	22	43	63
			P388-D1	5	10	21	34
	2	94‖	P815	5	11	36	55
			P815 + anti-Lyt2 MAB§	0	0	0	4.5
			P388-D1	4	11	23	47
			P388-D1 + anti-Lyt2 MAB§	0	0	2	4
			EL_4	0	0	0	0
Lyt2$^+$	1	99	P815	1	6.5	33	57
	2	97	P815	3	12	32	42
			P388-D1	3	6	15	27
L3T4$^+$	1	82¶	P388-D1	0	0	0	0
	2	80¶	P388-D1**	0	1	3	4

* Results of two representative experiments for each condition explored. ‡ C_3H lymphocytes injected to adult irradiated (C_3H × DBA)F_1 mice killed on day 6. P815 and P388 DI targets were syngeneic to DBA (H_2d). ‖ The isolated lymphocytes were 83% Lyt 2$^+$ and 13% T4$^+$. § Gut lymphocytes were incubated 30 min with anti-Lyt 2 MAB (at the concentrations used for immunofluorescence) before adding the target. ¶ Since GVHR elicited by selected L3T4$^+$ cells have a weak donor cell gut infiltration (Table I), the percentage of donor cells was increased by removing host cells by a panning procedure using an anti-DBA antiserum. ** P388 DI cells bear Ia molecules whose density could be increased by 48 h culture in IFN (13); this prior treatment of P388 DI cells was used in this experiment.

*Table V. In Vitro Lymphokine Release by Gut Lymphocytes from Mice with GVHR**

Lymphocytes used for GVHR induction	% Donor T cells‡ among gut lymphocytes	IL_3‖	IL_2§	IFN¶
Total	99	31	6	405
Lyt 2^+ subset	70	22	7	405
L3T4$^+$ subset**	34	56	36	405

* Average of two to three experiments. Supernatants obtained from 48-h cultures of 10^6 cells/ml with 2.5×10^6 Thy 1^+ depleted, 2,000-rad irradiated spleen cells from (C3H × DBA)F_1 mice. Except when mentioned, gut lymphocytes cultured without stimulating host cells did not release any activity in the culture medium.

‡ Host contamination among gut T lymphocytes was comparable to that shown in the experiments of Table I, except in GVHR induced by Lyt 2^+ subset, in which it was slightly higher.

‖ Activity tested on a IL_3-dependent cell line. The results are expressed as a percentage of ^{3}HTdR incorporation induced by an optimal amount of Wehi-3 supernatant (~30,000 cpm). The value shown was observed at 1/10 final concentration, and was the highest before decrease.

§ Activity tested on a IL_2-dependent cell line. Results expressed as for IL_3, in percentage of incorporation induced by an optimal amount of supernatant of concanavalin A–stimulated rat spleen cells (~55,000 cpm). The values shown were observed at 1/2 final dilution.

¶ Expressed in International Units.

** In cultures performed without the addition of stimulating host cells, IL3 activity in the supernatant was 10% of maximal stimulation at 1/5 final dilution. To evaluate the lymphokine release observed with this subset, one must take into account the fact that, in this condition, donor T cells represent only about 1/3 of the recovered gut T cells because of reduced infiltration.

prevents the acceleration of the epithelium renewal (Fig. 10). Second, when the localization and fate of donor T cells was studied at various days after the elicitation of GVHR in non-irradiated adult or newborn mice, the persistence or disappearance of parental T cells was always found to be associated with similar evolution of the gut lesions; in F_1 mice injected with parental selected L3T4$^+$ cells, disappearance of parental T lymphocytes in the spleen and in the gut is accompanied by disappearance of the intestinal lesions, while these mice suffer from a severe "systemic lupus erythematosous-like" form of chronic GVHR, as described by Rolink and Gleichmann (3). These results are in correlation with the observations that in humans suffering from chronic GVHR (which resembles autoimmune collagen vascular disease) the small bowel appears to be spared (25, 26).

The following points will be discussed now: (*a*) the subset composition of the lymphocytes infiltrating the gut; (*b*) the capacity of each subset to create epithelial damage; (*c*) the relationship between gut GVHR and histocompatibility antigens; and (*d*) the mechanisms of epithelial damage.

What is the subset composition of the gut T lymphocytes in GVHR elicited across various antigeneic differences, and what are the factors governing the degree of infiltration by each subset? In fully semi-allogeneic hosts, as well as in hosts differing only at class I MHC locus, the vast majority (about 4/5) of the donor T cells infiltrating the gut are Lyt2$^+$, while, in contrast, in hosts differing only at class II MHC locus and with a gut GVHR of comparable severity, gut T cells of donor origin are about evenly distributed between Lyt2$^+$ and L3T4$^+$ cells. This shows that donor T-cell subsets respond differently to different antigenic stimuli (a point we will discuss below). In addition, comparison of the subset composition of the gut infiltrate to that of blasts circulating in the TD (since it is known that the former originates from the latter [6]), and to that of donor blasts proliferating in the host LN, showed a striking difference: whatever the antigenic stimulus, and thus the extent of each subset proliferation, there was always a markedly higher proportion of L3T4$^+$ blasts in the TD than in the gut infiltrate or in the LN (Table I). This suggests that the L3T4$^+$ blasts have more tendency to circulate than Lyt2$^+$ blasts, but a lesser gut-homing tendency, a point that was directly confirmed by transfer experiments with selected TD blasts: Lyt2$^+$ TD blasts had indeed a much greater (about 5 times) gut-homing tendency. In conclusion, both donor T-cell subpopulations infiltrate the gut in GVHR, the extent of gut infiltration by a given subpopulation depending on two main factors: (*a*) the difference in properties of circulation and gut-homing of each subpopulation of blasts; and (*b*) the extent of the proliferation of each subpopulation, which depends on the nature of the antigenic stimulation (which we will discuss below).

Are both donor T-cell subsets equally capable of creating gut damage? The use of selected subsets of donor T cells shows that both subsets can lead to gut GVHR with epithelial damage (in condition excluding, by the study of the blasts recovered from the gut and TD, the possibility of an inadequate selection [Table I]). In irradiated mice, three times more Lyt2$^+$ than L3T4$^+$ cells are required to elicit epithelial damage of comparable intensity; but this may simply indicate that the presence of L3T4$^+$ cells is required for an optimal proliferation of Lyt2$^+$ cells. The higher efficiency of L3T4$^+$ cells to create epithelial damage could be deduced from another observation: in GVHR elicited by selected L3T4$^+$ cells, marked epithelial cell alterations were associated with a smaller, sometimes very weak T-cell infiltration; this was especially obvious in newborn mice (Table III). In non-irradiated adult mice, in which parental L3T4$^+$ lymphocytes were eliminated more easily and sooner than Lyt2$^+$ lymphocytes (probably because of their greater ability to induce anti-idiotypic host cells) (28), a very weak donor L3T4$^+$ cell infiltration in the gut wall was sufficient to induce epithelial alterations.

Is gut GVHR more related to a given antigenic stimulus acting on a given T cell subset? It is striking, as already mentioned, that differences in class II MHC locus preferentially stimulate donor L3T4$^+$ cells, when compared to differences in class I MHC locus or to a semi-allogeneic situation. It must be noted, however, that even in the case of antigenic differences restricted to class I or class II MHC locus, both subsets are induced to proliferate. Thus, it appears that there is not, as previously suggested (29), an absolute correlation between the surface phenotype of the parental T cells and their ability to be stimulated by class I or class II MHC antigens, but rather a preferential stimulation of L3T4$^+$ cells by class II and of Lyt2$^+$ cells by class I MHC antigens, which result agrees with a number of reports (30–33). That class II antigenic disparity does play a role in the induction of gut GVHR that is greater than class I MHC differences is obvious from the observation that gut lesions are easier to induce across class II MHC locus, as the elicitation of gut GVHR across class I MHC locus requires a high number of parental T lymphocytes (a condition which was not reached in newborn mice under the experimental conditions used, since no GVHR was observed). This observation agrees with the observations of Piguet (17). The strongest stimulation of L3T4$^+$

cells by class II MHC antigens, and the easy induction of strong GVHR across class II differences, correlate well with a greater ability of L3T4 cells infiltrating the gut wall to create damage (as we discussed above). We emphasize that a full histoincompatibility is more complex than the cumulation of class I and II MHC antigenic stimulations; minor histocompatibility antigens are also involved, and it has been shown that they stimulate $Lyt2^+$ cells (34): this fact could explain the $Lyt2^+$ predominance in the gut infiltrate in semi-allogeneic conditions.

What are the mechanisms of gut epithelial cells damage? Parental lymphocytes isolated from the gut mucosa of mice with semi-allogeneic GVHR display specific cytotoxicity against target cells bearing the MHC antigens of the other parent. However, two lines of evidence indicate that specific cytotoxicity by direct contact between donor T cells and host epithelium cannot be an exclusive, or even a major, mechanism of epithelial injury: (*a*) The cytotoxicity was almost completely eliminated by the presence of anti-Lyt 2 antiserum, indicating that it was restricted to Lyt 2^+ cells. Furthermore, donor $L3T4^+$ cells, obtained from the gut of F_1 mice injected with selected parental L3T4 cells, were not cytotoxic against target cells bearing Ia antigens, although these mice exhibit strong gut GVHR lesions. (*b*) Fetal gut grafts, syngeneic or allogeneic to the parental donor cells, show, during GVHR, comparable lesions (35, 36), with comparable infiltration by donor T cells originating in the host PP (6), and comparable increase of epithelial cell renewal (Fig. 11). The donor cells, when homing in on the syngeneic graft, are probably stimulated by Ia^+ macrophages or dendritic cells of the lamina propria, which derive from the host bone marrow (37) and are radioresistant. These results suggest that lymphokines released by stimulated parental cells, rather than direct specific cytotoxicity, may be responsible for the epithelial lesions, as proposed by MacIntosh et al. (36). Indeed, donor lymphocytes, isolated from the gut of mice undergoing a GVHR elicited with total T cells or selected subpopulations, released (when cultured in the presence of host cells) rather high levels of IFN and of IL3, as well as smaller amounts of IL2. Both donor Lyt 2^+ and $L3T4^+$ cells released comparable amounts of IFN, which is likely to be responsible for the increased expression of Ia on the epithelial cells (38), and thus, for further stimulation of donor cells. This also agrees with the appearance of Ia on various cell types, including gut epithelium, in rats injected with selected subsets of semi-allogeneic T lymphocytes (39). In contrast, IL3 release is consistently higher with $L3T4^+$ than with Lyt 2^+ cells, a fact which correlates well with the appearance of gut MCs in GVHR elicited in nonirradiated mice (since MC precursors are radiosensitive); MCs, which differentiate from precursors present in the gut wall under the effect of IL3 release (7, 40), are found in much higher numbers in GVHR elicited with $L3T4^+$ as compared to Lyt 2^+ parental cells in semi-allogeneic conditions, and also across class II MHC loci. However, if the accelerated epithelial renewal is indeed related to the effect of some lymphokine(s) preferentially released by $L3T4^+$ cells, it cannot be due to IL3 alone (41), since experiments using in vivo perfusion of recombinant murine IL3 (42) did not show any modification of the epithelial cell renewal (unpublished observation).

Finally, the arrival of donor T cells in the host mucosa has effects other than the elicitation of epithelial damage. There was a marked disappearance of IgA plasma cells, already noted in GVHR of mice (1) and man (43), which may be related to the general haematopoietic suppression, since it was closely paralleled by an haematocrit decrease (Table II), also observed in newborn mice with GVHR induced by selected L3T4 cells (Table III). On the other hand, gut GVHR leads to the appearance, detectable only in the newborn mice normally devoid of these cells, of host Lyt 2^+ intraepithelial granulated lymphocytes that did or did not bear the Thy 1 marker (14–16), i.e., of gut IEL appearing normally later in life (6). This differentiation from local precursors, which parallels MC differentiation (Table III), may similarly result from interleukin release by activated donor T cells. However, the inducing factor in the appearance of the characteristic granules of the gut mucosal lymphocytes appears to be the gut mucosal environment itself. Granules were observed both in donor cells, which were activated T cells developing granules only in the gut (Figs. 6 and 7), and in the peculiar Lyt 2^+ Thy 1^- cells derived from the host, whose thymic origin is unlikely, since similar cells are found in nude mice (14). Study of the ontogeny and role of the granulated IEL is in progress. These studies are and will be of special importance for the understanding of various intestinal diseases in addition to GVHR, in which common pathogenic pathways—involving T cells activated in the PP and seeding the gut wall, where they can be further stimulated by repeated contact with their antigens—may be involved.

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