Identity of Immune Cells in Graft-Versus-Host Disease of the Skin

Analysis Using Monoclonal Antibodies by Indirect Immunofluorescence

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The cellular infiltrate in skin biopsies of 9 patients with graft-versus-host disease (GVHD) has been characterized with the use of monoclonal antibodies by indirect immunofluorescence. Most infiltrating cells in dermis reacted with monoclonal antibodies which recognize T-cell antigens. A mean of 45% of all dermal cells were T11-reactive, while a smaller proportion of cells were identified by another "pan" antibody, OKT3. In all but two instances the proportion of dermal cells reactive with OKT8 exceeded the proportion reactive with OKT4. Anti-Tac, which identifies activated T cells, reacted with a variable proportion of cells. Monocytes and null cells (OKM1$^+$) were frequently observed but were less numerous than T-lymphocytes. Infiltrates were sparsely populated with OKT6-reactive cells, and there was no difference between the number of intraepidermal cells reactive with this antibody in study subjects and normal controls. Few cells reactive with Leu 7 (large granular lymphocytes) or with anti-B-cell reagents were seen. These findings may have clinical implications for use of monoclonal antibodies for prophylaxis and treatment of GVHD. (Am J Pathol 1984, 116:436–440)

GRAFT-VERSUS-HOST DISEASE (GVHD), affecting many organs, including the skin, liver, and gastrointestinal tract, is a major cause of morbidity and mortality in bone marrow graft recipients.[1] It occurs in patients who have received allogeneic marrow from a matched or mismatched donor and is thought to be generated by immunologic reaction between lymphocytes of the donor and tissues of the host.[2,3] Investigation of animal models suggests that T-lymphocytes and natural killer (NK) cells play a major role in the pathogenesis of GVHD.[3–5] Skin biopsy is regarded as a useful diagnostic technique.[7–9]

Monoclonal antibodies have been used to identify infiltrating mononuclear cell populations in prototypic cell-mediated processes such as human renal[9] and skin[10] graft rejection and cutaneous delayed-type hypersensitivity[11] as well as GVHD.[12] In this investigation, a series of monoclonal antibodies were used to identify and enumerate mononuclear cells infiltrating the skin in a well-characterized series of patients with GVHD. Our studies confirm prior observations that most identifiable infiltrating cells are T-lymphocytes, reactive with the monoclonal antibody OKT8.[12] In addition, we demonstrate that certain affected tissues have numerous infiltrating cells reactive with OKT4 as well as cells expressing receptors for interleukin 2.

Materials and Methods

Nine patients (ages, 4–29 years; 7 male, 2 female) with a clinical course and skin histologic features

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Table 1—Clinical Characteristics of GVHD in 9 Study Patients

Patient	Original disease*	Duration of graft (days)	Prophylaxis†	Extracutaneous involvement	Clinical grade‡	Histologic grade‡	Severity of infiltrate
1	ANLL	17	1	Liver	3	I	Moderate
		33	1		3	I	Moderate
		105	1			I	Moderate
2	ALL	24	1,2	Gut	1	I	Moderate
		34	3	Liver	1	I	Mild
3	ANLL	31	1,3,4	None	1	II	Moderate
4	LL	34	1,3,4	None	3	II	Moderate
5	CML	38	1,3,4	Liver	1	II	Severe
6	CML	38	1,3,4	Liver	3	II	Mild
7	ALL	68	1	None	1	I	Mild
8		160	1	Liver		I-II	Mild
9	ALL	314	1,3,4	Liver, mucosa		I-II	Moderate

* ANLL, acute nonlymphocytic lymphoma; ALL, acute lymphocytic leukemia; LL, lymphocytic lymphoma; CML, chronic myelogenous leukemia; IAA, idiopathic aplastic anemia.
† 1, methotrexate; 2, OKT3; 3, prednisone; 4, anti-thymocyte globulin.
‡ See Glucksberg et al[1] for criteria.

characteristic of GVHD were selected for the study. Clinical and pathologic features of their disease are summarized in Table 1. Tissues were obtained prior to specific therapy for GVHD, and all patients responded to therapy except Patient 1, who experienced a persistent rash and liver involvement.

A clinical grade of skin involvement was assigned with the use of the criteria of Glucksberg et al.[1] Light-microscopic analysis was performed on formalin-fixed tissue stained with hematoxylin and eosin and with toluidine blue. The severity of the infiltrate was semiquantitively graded as mild, moderate, or severe.

Immunofluorescence studies were performed on frozen tissue sections prepared as previously described.[9,11] Briefly, 4-μ-thick frozen sections were acetone-fixed, washed, and treated sequentially with appropriately diluted monoclonal antibodies (Table 2), fluorescein isothiocyanate (FITC)-conjugated $F(ab')_2$ rabbit anti-mouse IgG (heavy and light chains), and FITC $F(ab')_2$ goat anti-rabbit IgG (heavy and light chains) (Cappel Laboratories, Cochranville, Pa). The sections were then treated with ethidium bromide to stain nuclei and *p*-phenylenediamine to retard fluorescence fading.[13] In addition, sections of each tissue were reacted with a mixture of FITC-conjugated $F(ab')_2$ goat anti-human IgM (μ) and FITC $F(ab')_2$ goat anti-human IgD (δ) (Tago, Inc., Burlingame, Calif). Reactive dermal cells (apple-green fluorescent plasma membrane surrounding orange-red nucleus) and total dermal cells (all dermal cells having nuclei reactive with ethidium bromide) identified by indirect immunofluorescence were enumerated independently by two investigators. The number of reactive cells was expressed as a percentage of the total number of dermal cells as previously described.[11] In addition, the number of epidermal dendritic cells reactive with OKT6 and the number of basilar epidermal cells was determined in each tissue and in five normal skin tissues.[11] Results were expressed as reactive cells per 100 basilar epidermal cells. In an additional study, tissues were sequentially reacted with anti-Tac, tetraethylrhodamine isothiocyanate (TRIC)-conjugated goat anti-mouse IgG (γ) (Cappel), mouse serum, and FITC-labeled OKT8 (Ortho) or FITC T11 (Coulter).

Anti-mouse and anti-rabbit antisera were human plasma absorbed. Control sections of each tissue were

Table 2—Monoclonal Antibodies

Antibody*	Antigen	Reactivity with mononuclear cells in blood
TA-1	p 95/170	T-lymphocytes, monocytes
T11	Sheep erythrocyte receptor	T-lymphocytes (pan), some NK cells
OKT3	p 19	Mature lymphocytes
OKT8	p 76	Suppressor/cytotoxic lymphocytes
OKT4	p 62	Helper/Inducer lymphocytes
Anti-Tac	Interleukin 2 receptor	Activated T-lymphocytes
OKM1	iC3b-receptor (CR3)	Monocytes and some NK cells
Leu 7		Large granular lymphocytes
OKT6	p 45/12	Thymocytes
BA-1		B-lymphocytes, granulocytes
Anti and anti	Heavy chain Ig	B-lymphocytes

* TA-1 and BA-1 were made at University of Minnesota. Anti-Tac was provided by T. Waldmann, NIH, Bethesda, Maryland; T11 was purchased from Coulter Immunology, Hialeah, Florida; OKT3, OKT8, OKT4, OKM1, and OKT6 were from Ortho Pharmaceutical Corp., Raritan, New Jersey; Leu 7 was from Becton Dickinson, Inc., Mountain View, California.

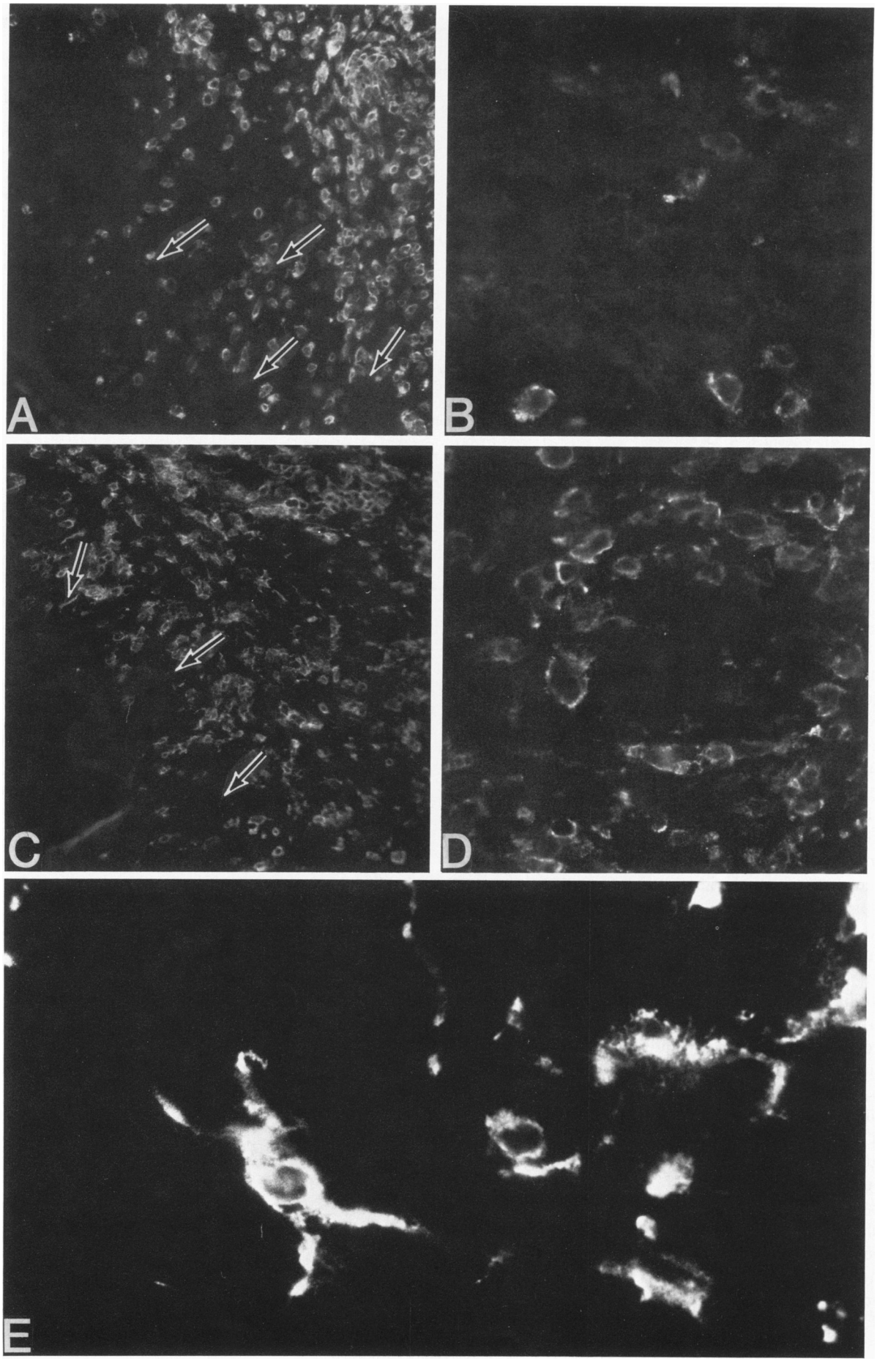
A
B
C
D
E

Table 3—Percentage of Dermal Cells Identified in Frozen Tissue Sections With the Use of Monoclonal Antibodies by Indirect Immunofluorescence

Patient	Graft duration (days)	TA-1	T11	OKT3	OKT8	OKT4	Anti-Tac	OKM1	Leu 7
1	17	7	13	10	5	8	1	3	<1
	33	21	56	15	11	4	2	15	9
	105	52	58	30	30	10	13		11
2	24		24	13	11	10	2		<1
	34		50	23	27	18	1	11	<1
3	31	65	10	20	16	6	2	40	<1
4	34	56	61	36	52	4	3	4	5
5	35	74	31	35	36	13	3	63	<1
6	38	87	75	28	35	6	7	7	<1
7	68		61	17	18	12	2	19	5
8	160	23	28	17	7	3	2	10	5
9	314	19	18	20	10	13	3	8	<1

reacted with the 2 fluorochrome layers described above without prior application of a monoclonal antibody. In addition, heavily infiltrated tissues were reacted with mouse myeloma-derived antibodies of the same immunoglobulin subclasses as the hydridoma-derived antibodies used, followed by a double fluorochrome layer. Studies comparing anti-Tac and OKT8 reactivity were controlled by sequential application of $F(ab^1)_2$ TRIC goat anti-mouse IgG, mouse serum and FITC OKT8 to sections of NZB/NZW mouse kidney tissue. Control sections revealed no evidence of nonspecific binding by monoclonal antibodies or by heteroantisera.

Results

Twelve study tissues were obtained from 9 patients (Table 1). Light-microscopic analysis of all tissues revealed dyskeratosis, satellite cell necrosis, and degeneration of the basal layer, characteristic of GVHD.[8] No tissues had lupus-like, poikiloderma-like, lichen planus-like, or sclerodermoid lesions of chronic GVHD.

Immunofluorescence studies revealed that most identifiable infiltrating cells in the dermis were T-lymphocytes ($OKT3^+$, $T11^+$; Figure 1, Table 3); however, fewer cells reacted with OKT3 than with T11 (Table 3). Analysis of T-lymphocyte subpopulations revealed that in all but two instances (Patients 1 and 9) the proportion of dermal cells reactive with OKT8 exceeded the proportion reactive with OKT4 (Table 3). Anti-Tac identified a variable proportion of dermal cells, which constituted 2.4–23% of the $T11^+$ population. Double-labeling studies revealed that few of the cells reactive with anti-Tac were also reactive with OKT8. Monocytes and null cells ($OKM1^+$) were frequently observed but less numerous than T-lymphocytes (Figure 1, Table 3). In most tissues dermal infiltrates were sparsely populated with OKT6-reactive cells; however, this antibody did identify 2% of dermal cells in tissue from Patient 1 (105 days), 4% in Patient 8, and 7% in Patient 9. OKT6 reacted with a mean of 4 cells per 100 basilar keratinocytes (range, 0–13) in study tissues (Figure 1) and 6 (range, 1–14) per 100 basilar keratinocytes in controls. Most tissues contained few cells reactive with Leu 7. Mature B-lymphocytes ($BA\text{-}1^+$, IgD^+, IgM^+) were rarely seen (data not shown).

GVHD developed in 1 patient (2) to whom OKT3 antibody had been administered. That patient's tissue contained substantial numbers of cells reactive with that antibody. His peripheral blood at that point was not analyzed because the small number of white cells did not permit enumeration.

There was no apparent correlation between mononuclear cell populations identified *in situ* and duration of the marrow allograft or severity of microscopic or clinical changes in the skin.

Discussion

Graft-versus-host disease is the result of an immune response to engraftment of alloreactive hemopoietic cells. Although the pathogenesis is not fully understood, investigation of animal models and *in vitro* analyses of human cells mediating alloreactivity suggest that specific cytotoxicity and natural killer activity may have a pathogenetic role in GVHD.[2–5] Monoclonal antibodies have recently been used to identify cell populations *in situ* in human renal[9] and

Figure 1—Immunofluorescence photomicrographs demonstrating inflammatory cells identified with monoclonal antibodies in dermis (**A–D**) and epidermal Langerhans cells (**E**) in skin tissue from patients with GVHD. **A**—T11. (×150) **B**—OKT4. (×750) **C**—OKT8. (×150) **D**—OKM1. (×600) **E**—OKT6. The *arrows* denote the dermal-epidermal junction. (×1150)

skin graft rejection[10] and delayed-type hypersensitivity[11] as well as in GVHD.[12]

Our study demonstrates that most cells infiltrating the dermis in GVHD are identified by monoclonal antibodies (OKT3, T11, TA-1) which recognize T-lymphocytes. Of these T-phenotype cells fewer appear to bind OKT3 than T11. Whether some ($OKT3^+$, $T11^+$) cells bear a thymocyte phenotype ($OKT4^+$, $OKT8^+$) is not known. The relatively high ratio of OKT8 to OKT4-reactive cells is also of interest, because cytotoxic effector cells responding to Class I major histocompatibility alloantigens are generally recognized by OKT8.[14] On the other hand, the role of OKT4-reactive cells is not clear. Some may function as helper cells, while others may exert cytotoxicity directed at Class II MHC alloantigens.[14] It is interesting that most cells bearing the interleukin 2 receptor (anti-TAC^+) were not reactive with OKT8. Whether these cells are activated $OKT4^+$ T-lymphocytes or NK cells is not known.

OKM1, which identifies monocytes as well as cells capable of exerting NK activity,[15] reacted with a substantial proportion of infiltrating cells in 8 of 10 tissues. Leu 7, which recognizes some peripheral blood NK cells,[16] identified few cells in our study tissues. Taken together, these data would suggest that mononuclear phagocytes, but not NK cells, have an important effector role in GVHD or that NK cells undergo modulation of surface antigens at the target tissue.

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