Identification of Natural Killer (NK) Cells in Lesions of Human Cutaneous Graft-Versus-Host Disease: Expression of a Novel NK-Associated Surface Antigen (Kp43) in Mononuclear Infiltrates

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We performed an immunohistochemical analysis of skin biopsies from 13 allogeneic bone marrow transplant (BMT) recipients, undergoing either acute graft-versus-host-disease (aGVHD, n = 8) or chronic GVHD (cGVHD, n = 5). A panel of different monoclonal antibodies (MoAb) was employed including anti-CD2, -CD3, -CD4, -CD8, -CD11b, -CD16, -CD56, and -CD57, as well as a recently described reagent (HP-3B1) specific for a novel natural killer (NK)-associated cell-surface antigen (Kp43). Our data indicate that in aGVHD lesions the proportions of $CD2^+$ cells often exceeded those detected with anti-CD3 MoAb. Double labeling confirmed the presence of $CD2^+ CD3^-$ lymphocytes and suggested the coexpression in some cells of CD2 and CD11b. When MoAb specific for non-lineage-restricted NK-associated markers were employed, anti-CD56 and -CD57 occasionally stained variable numbers of lymphocytes ($\bar{x}$ = 14.6% of mononuclear cells in 0.05 mm^2, range <1–48% and $\bar{x}$ = 10.3%, range 2–25%, respectively), whereas no CD16+ lymphocytes were observed. In contrast, most samples consistently displayed substantial proportions of Kp43+ cells ($\bar{x}$ = 32.8%, range 12–63%), which appeared CD3− and were mainly located at the dermoepidermal junction. On the other hand, sections from most (four of five) cGVHD lichenoid lesions analyzed displayed lower proportions of Kp43+ and CD56+ cells. Our data point out the interest of the anti-Kp43 MoAb to identify NK cells in aGVHD lesions, suggesting their pathogenetic participation. *J Invest Dermatol 97:659–666, 1991*

Donor-derived T lymphocytes are regarded as the effector cells responsible for the development of acute graft-versus-host disease (aGVHD) in patients undergoing HLA-identical allogeneic bone marrow transplantation (BMT). Supporting this notion it has been shown that pre-transplant T-cell depletion of the bone marrow (BM) inoculum does reduce the incidence of aGVHD [1], as predicted from previous studies in animal models [2]. In addition, immunohistochemical analysis of mononuclear infiltrates from aGVHD lesions, employing monoclonal antibodies (MoAb) specific for cell-surface differentiation antigens, have revealed the presence of CD3+ CD2+, CD8+ cells, as well as lower proportions of CD4+ lymphocytes [3–10]. On the other hand, data derived from experimental BMT models in mice suggest that natural killer (NK) cells may participate in the pathogenesis of GVHD [11–14]. So far, studies in clinical aGVHD have lent little corroboration to the role of NK cells in lesions. Although the presence of some infiltrating cells expressing non-lineage-restricted surface antigens, such as CD11b or CD57 (HNK-1), has been occasionally reported [5,15], by using MoAb specific for the CD16 surface marker the participation of NK cells has been dismissed in other studies [9,10].

Recently, we obtained a MoAb specific for a novel functional cell-surface antigen, designated as Kp43 [16], whose expression appears restricted to human NK cells, γ/δ + lymphocytes, as well as to minor subsets of α/β + CD56 + T cells. The Kp43 antigen is strongly induced on most in vitro activated NK cells and, interestingly, the specific MoAb is capable of inhibiting their interleukin-2 (IL2)-dependent proliferative response.

In the present study we have carried out an immunohistochemical analysis of skin biopsies from 13 allogeneic BMT recipients undergoing either acute (n = 8) or chronic (n = 5) GVHD. A panel of different MoAb, including the anti-Kp43 reagent, was used either in single or double immunoenzymatic labeling methods. Our results indicate that aGVHD lesions are often infiltrated by substantial proportions of mononuclear cells that display phenotypic characteristics of NK cells, namely the presence of the Kp43 antigen in CD2 + CD3− cells. In contrast, most chronic GVHD lesions analyzed included lower proportions of Kp43 + cells, and mononuclear infiltrates were predominantly CD3 + CD8 +. Altogether, our

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Abbreviations:

- aGVHD: acute graft-versus-host disease
- APAAP: alkaline phosphatase–anti-alkaline phosphatase
- BM: bone marrow
- BMT: bone marrow transplantation
- CD: cluster of differentiation
- cGVHD: chronic graft-versus-host disease
- DAB: 3-3′ diaminobenzidine tetrahydrochloride
- IIP: indirect immunoperoxidase
- IL-2: interleukin 2
- MoAb: monoclonal antibody
- NK: natural killer
- TBS: tris-buffered saline

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Table I. Summary of Clinical Data of Acute GVHD Patients[a]

Case	Age/Sex	Diagnosis	Onset of aGVHD[b]	Date of Biopsy[b]	Histology	Clinical Grade	Other Organs Involved	GVHD-Therapy Before Biopsy	Outcome	Follow-Up (Months)
1	27/M	CML-AF	+24	+25	III	II	Liver	No	R	30
2	13/M	CML-2ndCF	+24	+43	II	II	No	MP + ATG	R	50
3	18/M	ALL	+17	+44	II	IV	Liver	MP + ATG	R	35
4[c]	23/M	CML-CF	+35	+37	II	III	Liver	No	Progression to cGVHD	36
5	24/M	CML-CF	+45	+45	II	I	No	No	R	15
6	43/M	CML-CF	+14	+40	II	III	Liver, gut	MP	NR	3
7	23/M	SAA	+37	+41	II	II	Liver	MP	R	4
8	20/M	AML-M1	+17	+20	II	IV	Liver	No	NR	1,5
9[d]	44/F	AML-M5	+34	+35	II	I	No	No	R	11

[a] Abbreviations: CML, chronic myeloid leukemia; AF, acute phase; CF, chronic phase; ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; SAA, severe aplastic anemia; MP, methyl prednisolone; ATG, anti-thymocytic gamma-globulin; R, resolved; NR, not resolved.
[b] Day after BMT.
[c] Mismatched in one locus A.
[d] Autologous BMT patient.

data may contribute to a better understanding of the pathogenesis of aGVHD, pointing out the interest of the anti-Kp43 MoAb to identify NK cells in lesions.

MATERIALS AND METHODS

Patients and Biopsies A total of 13 biopsies from different allogeneic BMT recipients, 12 receiving marrow from an HLA-identical sibling and 1 (case number 4, Table I) from a sibling mismatched in one locus A, have been included in this study. All the cases have been submitted to the International Bone Marrow Transplantation Registry. Additionally, a single sample from an autologous BMT recipient displaying GVHD-like lesions was analyzed as well. A summary of the most relevant clinical features is included in Tables I (acute GVHD cases) and II (chronic GVHD cases). Conditioning therapy (fractionated total body irradiation and cyclophosphamide) and GVHD prophylaxis (methotrexate and cyclosporin A) were carried out as previously described [17,18].

Nine biopsies, obtained from allogeneic (n = 8) and autologous (n = 1) BMT patients between days +20 and +45 after BMT, displayed aGVHD lesions, consisting of a dermoepidermal mononuclear cell infiltrate, associated with apoptotic bodies located in the epithelium. According to the histologic criteria proposed by Lerner et al [19], severity of lesions varied from grade II (8 cases) to grade III (1 case). For the purpose of the present study, grade I lesions have not been included due to their nonspecific histologic features. Most cases showed focal infiltrates, separated by areas with no lesions. Five additional biopsies (three mucosal and two cutaneous), displaying lichenoid lesions, were obtained between days +174 and +300 after allogeneic BMT from different patients undergoing cGVHD. In these cases a dermoepidermal or submucosal mononuclear infiltrate was observed associated with acanthosis, hyperkeratosis, and occasional apoptotic cells. Additionally, skin biopsies obtained from cases of lichen planus (n = 12), multiforme erythema (n = 2), and contact dermatitis (n = 4) were studied.

Monoclonal Antibodies For immunohistochemical analysis a panel of different MoAb, specific for well-defined cell-surface differentiation antigens, was used (see Table III). In addition, we included the HP-3B1 MoAb specific for a novel NK-associated surface antigen (Kp43), which was originally described in our laboratory [16]. In all experiments control slides were included using ascitic fluid from the P3X63 myeloma as the primary antibody.

Immunohistochemical Methods Biopsies were taken and frozen by routine methods. Two different techniques were applied for staining tissue sections.

Indirect Immunoperoxidase (IIP): As previously described [26], acetone-fixed tissue sections (4 μm thick) were incubated with every MoAb for 30 min. Subsequently, slides were incubated with a peroxidase-conjugated rabbit anti-mouse IgG (Dako). Peroxidase deposition was developed using hydrogen peroxide and 3-3′ Diaminobenzidine (DAB, Sigma). Sections were counterstained with Carazzi's hematoxylin, dehydrated, and mounted by routine methods.

Double Immunoenzymatic Staining: In order to more accurately evaluate the presence of different cell subsets in the lesions, double immunoenzymatic labeling was carried out by combining different MoAb. As previously described [27], we applied the sequential method proposed by Mason et al [28]. First, the sections were stained by indirect immunoperoxidase (see above). Thereafter, they were saturated with nonspecific mouse Ig, washed in Tris-buffered saline, pH 7.6 (TBS), and sequentially incubated with a second MoAb, a rabbit anti-mouse IgG (Dako), and an alkaline phosphatase anti-alkaline phosphatase (APAAP) complex (Dako). The alkaline phosphatase reaction was developed by incubating sections with

Table II. Summary of Clinical Data of Chronic GVHD Patients[a]

Case	Age/Sex	Diagnosis	aGVHD	cGVHD: Day of Onset	Clinical Grade	Other Organs Involved	GVHD Therapy Before Biopsy	Outcome	Follow-Up (Months)
10	33/F	ALL-L2	Yes	+260	Mild	Oral mucosa eyes, liver	No	R	61
11	34/M	AML-M3	No	+180	Moderate	Liver, skin, oral mucosa	No	NR	20
12	17/M	SAA	Yes	+135	Mild	Oral mucosa	No	NR	31
13	38/M	NHL-LB	No	+125	Severe	Liver, skin, oral mucosa	MP CsA	NR	29
14	45/F	SAA	No	+170	Mild	Liver, oral mucosa	No	NR	14

[a] For abbreviations see Table I. NHL-LB, non-Hodgkin lymphoma, lymphoblastic type; CsA, cyclosporin A.

Table III. Monoclonal Antibodies

MoAb	CD	Cell Distribution[a]	Source[b]	Reference
TS2/18	CD2	T and NK cells	[c]	[20]
DAKO T3	CD3	Mature T cells	DAKO	
HP 2/6	CD4	T-cell subset, macrophages	HP	[21]
B 9.4	CD8	T- and NK-cell subsets	[c]	[22]
BEAR 1	CD11b	Macrophages, NK cells	[c]	[23]
Leu 11	CD16	NK cells, macrophages	B-D	
B73.1	CD16	idem	[c]	[24]
Leu 19	CD56	NK cells and minor T-cell subsets	B-D	
HNK 1	CD57	idem	[c]	[25]
HP 3B1		idem	HP	[16]

[a] Only the common cell distribution among mononuclear cells is mentioned.
[b] HP, Prepared from the original hybridomas at the Hospital de la Princesa (Madrid, Spain); B-D, Supplied by Becton-Dickinson (Mountain View, CA); DAKO, Supplied by Dako (Copenhagen, Denmark).
[c] TS2/18, B 9.4, BEAR 1, B73.1, and HNK 1 were kindly supplied by Drs. F. Sánchez-Madrid, B. Malissen, J. de Vries, B. Perussia, and the American Type Culture Collection (ATCC), respectively.

TBS, pH 8.4, containing Naphtol AS-MX phosphate (Sigma) and Fast Blue salt (Sigma) as the reaction substrate, as well as levamisole (Sigma) as an inhibitor for endogenous alkaline phosphatase. Sections were mounted in buffered gelatin-glycerol (Glycergel, Dako) for microscopic examination. As discussed [28], it has to be stressed that the results obtained with this technique are highly reliable when the pairs of MoAb employed stain different cell subsets, enabling their simultaneous detection. On the other hand, the coexpression of both antigens on the same cell population can only be suggested.

Slides were examined separately by two different observers. As previously described [29], the number of cells positively stained with every MoAb and the total number of mononuclear cells were counted by using a reticulate ocular. Results are expressed as the percentage of positive cells per 0.05 mm² tissue. In all cases only areas with well-developed lesions corresponding to the dermoepidermal zone were evaluated.

RESULTS

Immunohistochemical Analysis of aGVHD Skin biopsies from eight allogeneic BMT patients undergoing aGVHD were studied (Table I). Immunoperoxidase staining of tissue sections was performed with a panel of different MoAb, and the results are summarized in Table IV. CD2+ and CD3+ cells were detected in all cases, and the relative proportions of the former outnumbered CD3+ lymphocytes in most cases (Table IV and Figs 1*A*, *B*). To better substantiate the dissociation between the expression of both markers, we performed a double immunoenzymatic labeling. Sections were stained by immunoperoxidase with anti-CD3 MoAb, and subsequently labeled with anti-CD2 by the APAAP method. Such an approach confirmed unequivocally the presence of variable proportions of CD2+ CD3− cells in all cases, constituting in one instance (Table IV, case number 4, Fig 2*A*) the predominant phenotype. In agreement with previous reports [3–10], substantial proportions of CD8+ cells were observed, whereas a complex staining pattern was detected with anti-CD4 MoAb (not shown). In fact, some small round CD4+ cells were found, together with an overlapping population of large macrophage-type CD4+ cells. The expression of CD4 by macrophages has been previously well defined [30]. A similar complex pattern was observed with an anti-CD11b MoAb, which stained a population of macrophages, as well as variable proportions of small round cells (Fig 1*C*).

We addressed whether some CD2+ lymphocytes might coexpress CD11b, and double immunoenzymatic labeling allowed us to support this presumption. As shown in Fig 2*B*, the proportions of CD11b− CD2+ cells (stained in blue) were considerably lower than the total numbers of CD2+ cells detected in the same case (Fig 1*B*). In all instances we observed a partial overlap of both markers, which was more suggestive in biopsies where the dissociation between CD2 and CD3 expression was clearly substantiated. The data indirectly suggested that CD2+ CD3− cells might correspond to CD2+ CD11b+ lymphocytes.

Because the CD2+ CD11b+ and the CD2+ CD3− phenotypes have been ascribed to NK cells, we directly analyzed the expression of different non–lineage-restricted surface markers, preferentially expressed among mononuclear cells by NK lymphocytes. To this end we employed for immunoperoxidase staining anti-CD16, CD56, and CD57 MoAb, as well as the HP-3B1 MoAb, which

Table IV. Expression of Leukocyte Differentiation Antigens in GVHD Mononuclear Infiltrates[a]

Case Number	Type	Kp43	CD2	CD3	CD4[b]	CD8	CD56	CD57
1	A	29	74	55	42	58	9	21
2	A	38	69	52	32	45	8	2
3	A	25	67	41	39	49	8	25
4	A	63	77	34	48	30	48	8
5	A	35	68	49	33	50	15	8
6	A	12	65	60	49	61	<1	4
7	A	19	75	63	56	63	7	7
8	A	42	73	54	54	57	21	8
9[c]	A	12	57	58	62	35	<1	1
10	C	10	78	75	32	54	2	1
11	C	8	78	73	25	70	1	1
12	C	18	70	68	53	35	4	16
13	C	7	67	69	50	46	<1	11
14	C	30	77	79	57	54	24	23

[a] Tissue sections from acute (A) or chronic (C) GVHD lesions were stained by an indirect immunoperoxidase technique (IIP) with different MoAb. A quantitative estimation of the proportions of stained cells in mononuclear infiltrates was performed as described in *Materials and Methods*. The results are expressed as the percentages of positive cells referred to as the total number of mononuclear cells counted in 0.05 mm².
[b] The proportions of CD4+ cells include both large macrophage-type and small round cells.
[c] This sample corresponds to an aGVHD-like lesion from an autologous BMT.

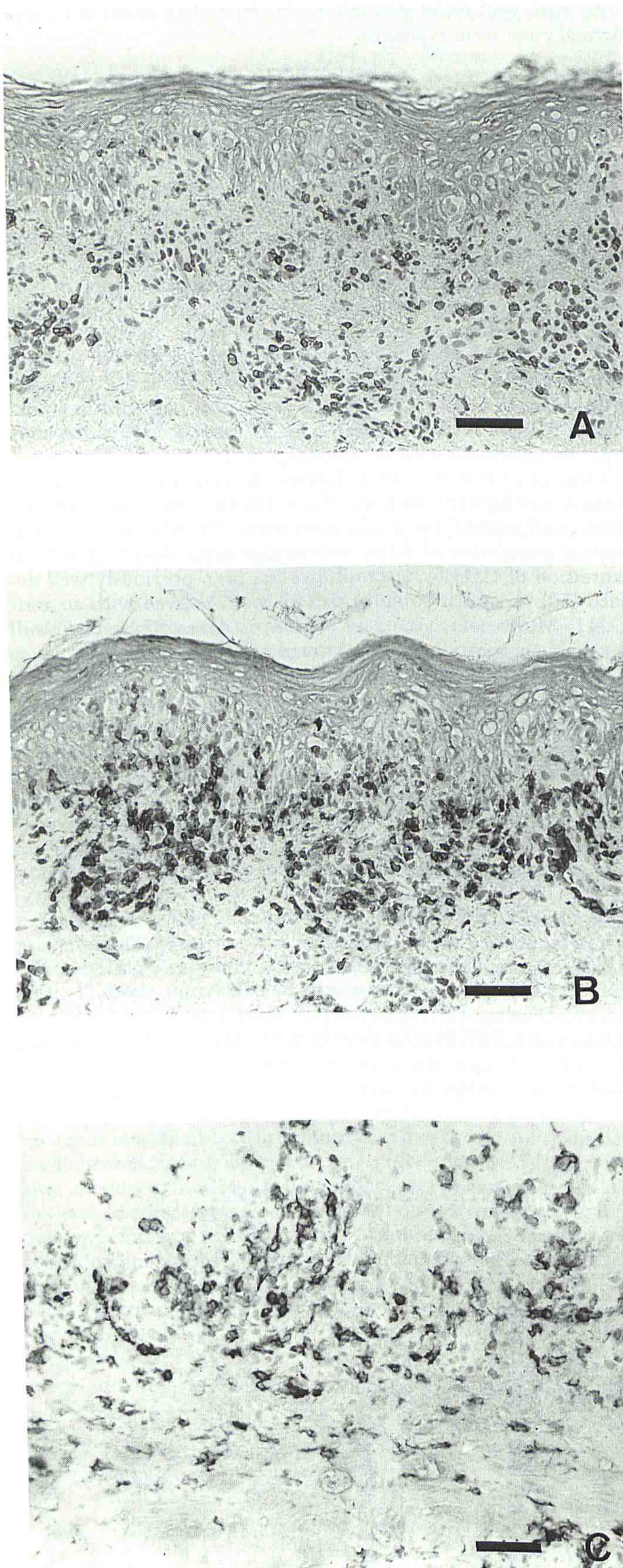


Figure 1. Expression of CD2, CD3, and CD11b in mononuclear infiltrates from aGVHD lesions. Skin sections from aGVHD lesions were stained by IIP with anti-CD3 (*A*), anti-CD2 (*B*), and anti-CD11b (*C*). *A* and *B* correspond to case number 4 (Table III) and *C* to case number 8. Magnification ×100. *bar*, 100 μm.

recognizes a novel NK-associated cell surface antigen (Kp43). No staining with either the B73.1 nor the Leu11 anti-CD16 MoAb was observed (not shown). CD57+ cells were poorly represented in all but two cases (Table IV, Fig 3*A*); some CD56+ cells were detected in seven of eight specimens, constituting only in three (Fig 3*B*, Table IV) a significant proportion (15–48%) of the infiltrates. In contrast to these results, anti-Kp43 MoAb consistently stained in most cases substantial proportions of small cells (Fig 3*C*, Table IV). Double immunoenzymatic staining indicated that the majority of Kp43+ cells were CD3− (Figs 2*C, E*), though the resolution of the technique did not allow us to entirely exclude the presence of some CD3+ Kp43+ cells (see Materials and Methods). In most instances Kp43+ cells were located near the dermoepidermal junction or even permeating the basal layer (Fig 2*D*); occasionally they formed small clusters around epidermal cells. It is noteworthy that the Kp43 expression was variable (Table IV); in one sample the Kp43+ population accounted for more than 50% of the infiltrate (Table IV, case number 4, Fig 2*C*), in five cases it ranged between 25 and 50% (Table IV, cases number 1, 2, 3, 5, and 8, Figs 2*E* and 3*C*), and in two biopsies it was under 20% of the total mononuclear cells (Table IV, cases 6 and 7). As shown in Fig 2*F*, double staining with anti-CD2 and Kp43 MoAb suggested that both markers overlap on the same population, although the presence of some Kp43+ CD2− cells cannot be entirely excluded. In two cases, double labeling with anti-Kp43 and anti-CD56 did not reveal the presence of CD56+ Kp43− cells (not shown).

Following the same approach, a biopsy derived from an autologous BMT recipient with aGVHD-like lesions was studied (Table IV, case number 9). The phenotypic characteristics of the infiltrating cells were comparable to those described above in allogenic BMT; however, scanty proportions of Kp43+ cells were detectable, as in case number 6.

Analysis of Chronic GVHD and Miscellaneous Dermatoses Biopsies from five allogeneic BMT patients who presented either mucosal (n = 3) or cutaneous (n = 2) lichenoid lesions were analyzed. The CD2+ CD3+ phenotype was predominant (Table IV, Fig 4*A*), contrasting with the findings in aGVHD. The numbers of CD8+ cells exceeded those of small round CD4+ cells. Only in a single case (Table IV, case number 14) Kp43+ cells were represented comparably to aGVHD lesions and a significant expression of CD56 and CD57 was also detected in that sample. In the remaining biopsies the Kp43+ population constituted a minority of the infiltrate (Fig 4*B*).

Skin biopsies from a group of miscellaneous skin disorders (see *Materials and Methods*) were studied with the anti-Kp43 MoAb and IIP. Lichen planus and multiforme erythema were selected because of their histopathologic similarities with GVHD whereas contact dermatitis was included as a typical T-cell–mediated process. The proportions of Kp43+ cells detected in the inflammatory infiltrates were in most cases insignificant, pointing out a clear difference with the characteristics of aGVHD lesions. Nevertheless, further studies are required to evaluate the possible participation of Kp43+ cells in the infiltrates from other dermatoses.

DISCUSSION

In the present study we report the detection of cells displaying an NK-associated phenotype in lesions from cutaneous aGVHD. By using a MoAb specific for a novel cell-surface antigen (Kp43), selectively expressed in NK cells and small subsets of T lymphocytes, we observed in most cases that substantial proportions of infiltrating mononuclear cells were Kp43+. Two-color immunohistochemical analysis revealed that the large majority of Kp43+ lymphocytes were CD3−. Moreover, the identification in some cases of CD56+ lymphocytes and of CD2+ cells that coexpressed CD11b further supports the presence of NK cells in aGVHD lesions. In contrast to the results obtained with the anti-Kp43 MoAb, anti-CD16 MoAb, which preferentially react with NK lymphocytes among peripheral blood mononuclear cells, did not stain the infiltrates, confirming other reports [9,10]. In most samples few CD57+ lymphocytes

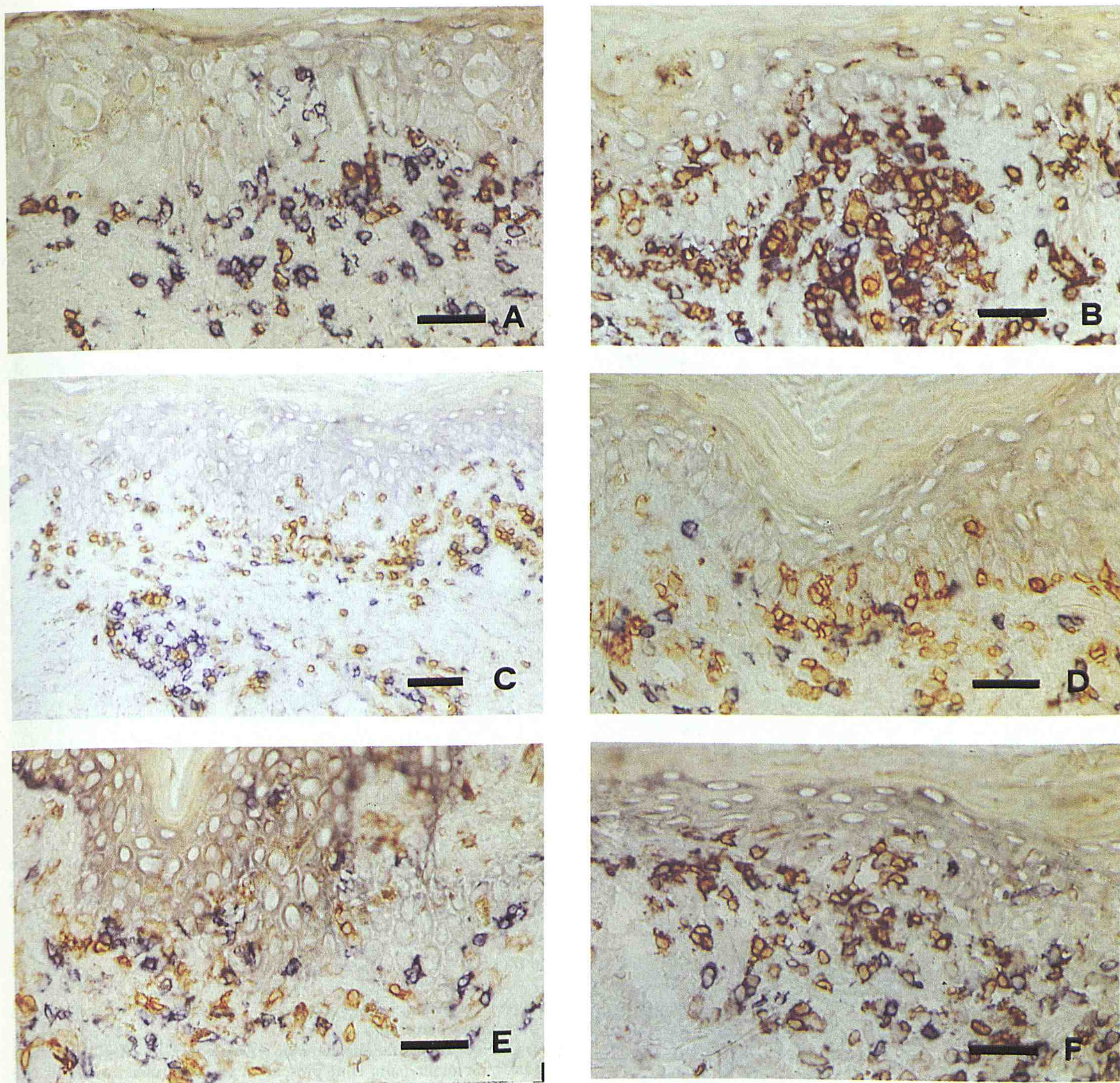
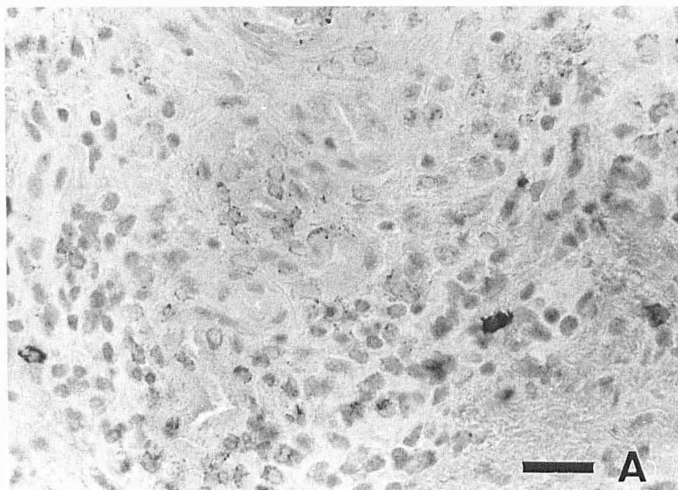
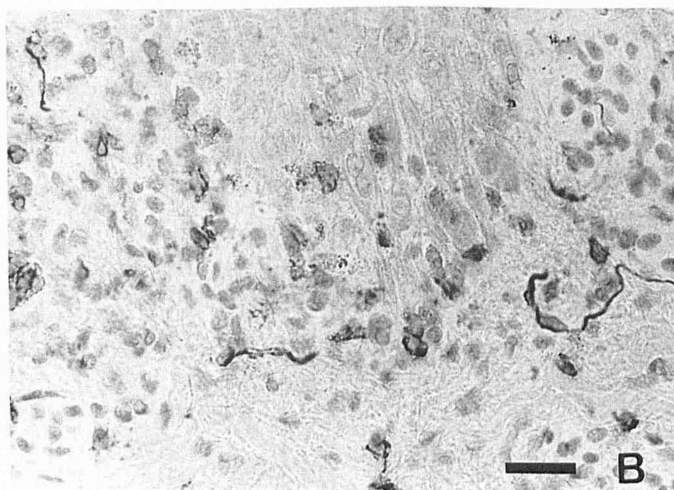


Figure 2. Double immunoenzymatic staining of mononuclear infiltrates from aGVHD lesions. Skin sections were sequentially labeled by IIP (brown stain) and the APAAP method (blue stain) employing the following combination of MoAb in this order: CD3/CD2 (*A*), CD2/CD11b (*B*), anti-Kp43/CD3 (*C, D, E*), anti-Kp43/CD2 (*F*). Sections correspond to cases number 4 (Table III) (*A, B, C, D,* and *F*) and case number 8 (*E*). Notice in *A* that many cells display only CD2 staining (blue); in *B*, a minority of mononuclear cells are CD11b− CD2+ (compare with Fig 1*B* from the same case). In *C–E* Kp43+ cells appear CD3−, contrasting with CD3+ lymphocytes stained in blue. In *F*, few cells appear stained only with anti-CD2 (blue). Magnifications ×100 (*C*) and ×200 (*A, B, D, E,* and *F*); *bars*, 100 μm and 60 μm, respectively.

were observed, and anti-CD56 MoAb only occasionally stained significant proportions of cells, although to a lesser extent than anti-Kp43. Several interpretations may be proposed to explain the dissociated expression of the different markers. First, the epitopes recognized by some MoAb either may not be fully exposed in tissue-infiltrating NK cells or may be affected during the fixation step. The lack of reactivity of some anti-CD16 MoAb in tissues has been previously described [31]. Alternatively, the antigens may be absent or faintly expressed due to the stage of differentiation/activation of NK cells, as reported for CD57, which is lost during NK cell activation [13].

GVHD-like lesions are occasionally observed in patients undergoing autologous BMT [32], and their pathogenesis is still unknown. We analyzed tissue sections from a single case of autologous BMT presenting aGVHD-like lesions and we detected the presence of some Kp43+ cells in the infiltrates, suggesting that NK cells in GVHD lesions are not necessarily related to the allogeneic situation. Although it is noteworthy that the proportion of Kp43+ cells was





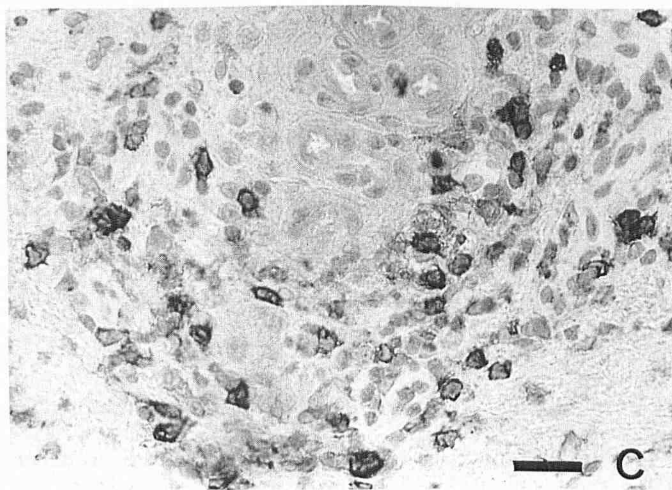
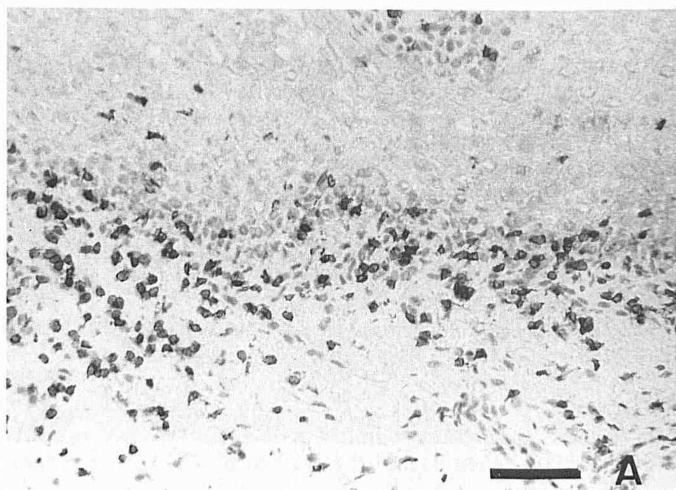


Figure 3. Differential expression of NK-associated surface antigens in mononuclear infiltrates from aGVHD. Serial sections from a single case (number 8, Table II) were stained by IIP with anti-CD57 (*A*), anti-CD56 (*B*), and anti-Kp43 (*C*). Anti-CD56 MoAb clearly stain neural dermal structures near the dermoepidermal junction. Magnification ×400; *bar*, 25 μm.



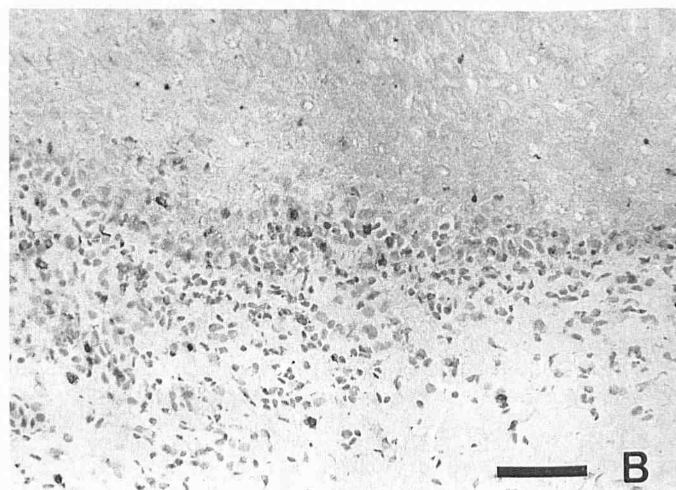


Figure 4. Comparative analysis of the expression of CD3 and Kp43 surface antigens in mucosal chronic lichenoid GVHD. Serial sections of oral mucosa from case number 10 (Table III) were stained by IIP with anti-CD3 (*A*) and anti-Kp43 (*B*) MoAb. Magnification ×100; *bar*, 60 μm.

lower than in most allogeneic BMT, further studies are required to draw any conclusions.

The pathogenetic mechanism of aGVHD in MHC-identical donor/recipient pairs is at present not fully understood, and the precise nature of the antigenic determinants, recognized in the inductive phase of the process, remains elusive. Regarding effector cells, T lymphocytes are considered to play a crucial role [1–10]. On the other hand, the possible participation of NK cells in GVHD has been also suggested in animal models [11–13]. On the basis of such observations and on our own data the following questions deserve special attention. First, is the presence of NK cells in lesions primary, thus implying an ability to selectively interact with tissue antigens? As an alternative, are they attracted to the infiltrates, subsequently to the activity of antigen-specific T lymphocytes that initiate the process? Finally, in any case, do NK cells play an active role in the development of lesions? Concerning the first point, different investigators have proposed that NK cells may selectively recognize surface structures in allogeneic cells. The role of NK cells in the F1-hybrid resistance phenomenon, and their ability to specifically discriminate Hh alloantigens in mice have been supported by several studies [33,34]. Moreover, an apparent selectivity of human NK clones to kill normal allogeneic cells has been recently reported

[35]. In spite of this data no clonally restricted polymorphic structures, related to the current concept of an antigen receptor, have been so far identified on NK cells. Thus, their hypothetical participation in specific recognition of antigenic molecules during aGVHD still rests on speculative ground.

An alternative, and more likely, explanation for the presence of NK cells in GVHD lesions is that they may be secondarily attracted to the infiltrates initiated by antigen-specific T cells, as previously suggested in an experimental model [12]. The recruitment of either mature NK cells or their precursors in GVHD lesions could be favored by the fact that increased proportions of NK cells, which behave functionally as activated, can be detected circulating early after BMT. In several studies this observation has not been correlated with the incidence of GVHD and it is considered to simply reflect the faster kinetics of NK cell recovery, as compared to other lymphoid lineages [36]. However, in other reports a relationship between the local increased NK activity and GVHD has been noticed [14,37].

Regardless of the nature of the mechanisms leading to the afference of NK cells to GVHD infiltrates, some indications indirectly support the notion that they may coparticipate in the pathogenesis of lesions [13]. In H-2-matched rodents GVHD has been prevented by an anti-asialo GM-1 antiserum [38], although such experiments are inconclusive because that treatment may also recognize activated T cells [39]. In a different model, BM cells derived from bg/bg NK-deficient mice failed to induce severe GVHD [40]. Moreover, activated NK cells are capable of synthesizing cytokines such as γ-interferon and tumor necrosis factor [13], which have been proposed to contribute to the pathogenesis of lesions [41,42], together with direct cell-mediated cytotoxicity [43].

In contrast to the clear expression of Kp43 in aGVHD infiltrates, most chronic GVHD lesions analyzed contained much lower proportions of Kp43+ cells and the predominant phenotype of mononuclear infiltrates was CD3+ CD8+, in agreement with other reports [29]. The scanty representation of NK cells in cGVHD infiltrates is compatible with the current opinion that such complication is mediated by newly differentiated T cells from the donor, which induce an autoimmune-like disorder [44].

The identification in GVHD infiltrates of cell-surface structures participating in the regulation of leukocyte function may have therapeutic implications. Recently, clinical trials have been conducted employing anti-IL2-receptor (CD25) MoAb in order to control aGVHD [45]. The observation that the anti-Kp43 MoAb inhibits the IL2-dependent proliferation of NK cells, without interfering with IL2 binding to its receptors [16], supports an important functional role for the molecule, which is at present being investigated. A better understanding of the biologic function of Kp43 could be considered of potential interest in the design of therapeutic trials.

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REFERENCES

1. Neudorf S, Filipovich A, Ramsay N, Kersey J: Prevention and treatment of acute graft-versus-host disease. Seminars in Hematology 21:91-100, 1984
2. Korngold R, Sprent J: Lethal graft-versus-host disease after bone marrow transplantation across minor histocompatibility barriers in mice. Prevention by removing mature T cells from mice. J Exp Med 148:1678-1684, 1978
3. Lampert IA, Suitters AJ, Janossy G, Thomas JA, Palmer S, Gordon Smith E: Lymphoid infiltrates in skin in graft-versus-host disease. Lancet II:1352, 1981
4. Lampert IA, Janossy G, Suitters AJ, Bofill M, Palmer S, Gordon Smith E, Prentice HG, Thomas JA: Immunological analysis of the skin in graft-versus-host disease. Clin Exp Immunol 50:123-131, 1982
5. Kaye VN, Neumann PM, Kersey J, Goltz RW, Balbridge BD, Michael AF, Platt JL: Identity of immune cells in graft-versus-host disease of the skin. A study using monoclonal antibodies by indirect immunofluorescence. Am J Pathol 116:463-440, 1984
6. Atkinsons K, Munro V, Vasak E, Biggs J: Mononuclear cell subpopulations in the skin defined by monoclonal antibodies after HLA identical sibling marrow transplantation. Br J Dermatol 114:145-160, 1986
7. Dreno B, Milpied N, Harousseau JL, Stalder JF, Bureau B, Milpied B, Barriere H: Cutaneous immunological studies in diagnosis of acute graft-versus-host disease. Br J Dermatol 114:7-15, 1986
8. Guyotat D, Mauduit G, Chouvet B, Kanitakis J, Vu Van H, Fiere D, Thivolet J: A sequential study of histological and immunohistological changes in the skin after allogeneic bone marrow transplantation. Transplantation 41:340-342, 1986
9. Paller AS, Nelson A, Steffen L, Gotschalk L, Kaizer N: T-lymphocyte subsets in the lesional skin of allogeneic and autologous bone marrow transplant patients. Arch Dermatol 124:1795-1801, 1988
10. Volc-Platzer B, Rappersberger K, Mosberger I, Hinterberger W, Emminger-Schmidmeier W, Radaskiewicz T, Wolff K: Sequential immunohistologic analysis of the skin following allogeneic bone marrow transplantation. J Invest Dermatol 91:162-168, 1988
11. Guillen FJ, Ferrara J, Hancock WW, Messadi D, Fonferko E, Burakoff SJ, Murphy GF: Acute cutaneous graft-versus-host disease to minor histocompatibility antigens in a murine model. Evidence that large granular lymphocytes are effector cells in the immune response. Lab Invest 55:35-42, 1986
12. Ferrara JLM, Guillen FJ, Van Dijken PJ, Marion A, Murphy GF, Burakoff SJ: Evidence that large granular lymphocytes of donor origin mediate acute graft-versus-host disease. Transplantation 47:50-54, 1989
13. Trinchieri G: Biology of natural killer cells. Adv Immunol 47:187-303, 1989
14. Prud'homme GJ, Bocarro DC: Natural supressor-like cells in local graft-versus-host disease. Cell Immunol 118:516-525, 1989
15. Dilly SA, Sloane JP: An immunohistological study of human hepatic graft-versus-host disease. Clin Exp Immunol 62:545-553, 1985
16. Aramburu J, Balboa MA, Ramírez A, Silva A, Acevedo A, Sánchez-Madrid F, O de Landázuri M, López-Botet M: A novel functional cell surface dimer (Kp43) expressed by natural killer cells and T cell receptor $\gamma/\delta+$ T lymphocytes. I: Inhibition of the IL-2 dependent proliferation by anti-Kp43 monoclonal antibody. J Immunol 144:3238-3247, 1990
17. Feig SA, Champlin R, Arenson E, Yale C, Ho W, Tesler A, Gale RP: Improved survival following bone marrow transplantation for aplastic anemia. Br J Haematol 54:509-517, 1983
18. Storb RH, Deeg HJ, Whitehead J, Appelbaum F, Beaty P, Bensinger W, Buckner CP, Clift R, Doney K, Farewell V, Hansen J, Hill R, Lum L, Martin P, McGriffin R, Sanders J, Stewart P, Sullivan K, Whitherspoon R, Yee G, Thomas ED: Methotrexate and cyclosporin compared with cyclosporin alone for prophylaxis of acute GVHD after bone marrow transplantation for leukemia. N Engl J Med 314:729-735, 1986
19. Lerner KG, Kao GF, Storb R, Buckner CD, Clift RA, Thomas ED: Histopathology of graft-vs-host reactions (GvHR) in human recipients of marrow from HLA matched sibling donors. Transpl Proc 6:367-371, 1974
20. Sánchez-Madrid F, Krensky AM, Ware CF, Robbins E, Strominger JL, Burakoff SJ, Springer TA: Three distinct antigens associated with human T lymphocyte-mediated cytolysis. Proc Nat Acad Sci USA 79:7489-7493, 1982
21. Carrera AC, Sánchez-Madrid F, López-Botet M, Bernabéu C, O de Landázuri M: Involvement of the CD4 molecule in a post-activation event on T cell proliferation. Eur J Immunol 17:179-186, 1987
22. Malissen B, Rebai N, Liabeuf A, Mawas C: Human cytotoxic T cell structures associated with expression of cytolysis. I. Analysis at the clonal cell level of the cytolysis-inhibiting effect of 7 monoclonal antibodies. Eur J Immunol 12:739-747, 1982
23. Keizer GD, Borst J, Figdor CG, Spits H, Miedema F, Terhorst C, De Vries JE: Biochemical and functional characteristics of the human leucocyte membrane antigen family LFA-1, Mo-1 and p150-95. Eur J Immunol 15:1142-1147, 1985
24. Perussia B, Acuto O, Terhorst C, Faust J, Lazarus R, Fanning V, Trinchieri G: Human natural killer cells analyzed by B73.1 a monoclonal antibody blocking FcR functions. II. Studies of B73.1 anti-

body-antigen interaction on the lymphocyte membrane. J Immunol 130:2142–2148, 1983

25. Abo T, Balch CM: A differentation antigen of human NK and K cells identified by a monoclonal antibody (HNK-1). J Immunol 127:1024–1029, 1981
26. Pulido R, Cebrián M, Acevedo A, O de Landázuri M, Sánchez-Madrid F: Comparative biochemical and tissue distribution study of four distinct CD45 antigen specificities. J Immunol 140:3851–3857, 1988
27. Sánchez-Mateos P, Cebrián M, Acevedo A, López-Botet M, Ortiz de Landázuri M, Sánchez-Madrid F: Expression of a GP33/27000 MW activation inducer molecule (AIM) on human lymphoid tissues. Induction of cell proliferation on thymocytes and B lymphocytes by anti-AIM antibodies. Immunology 68:72–79, 1989
28. Mason DY, Abdulaziz Z, Falini B, Stein H: Double immunoenzymatic labeling. In: Polack J, Van Noorden S (eds.). Immunocytochemistry. Wright PSG, Bristol, 1983, pp 113–128
29. Fujii H, Ohashi M, Nagura H: Immunohistochemical analysis of oral lichen planus like eruption in graft-versus-host disease after allogeneic bone marrow transplantation. Am J Clin Path 89:177–186, 1988
30. Stewart SJ, Fujimoto S, Levy R: Human T lymphocytes and monocytes bear the same Leu-3 (T4) antigen. J Immunol 136:3773–3778, 1986
31. Berti E, Soligo D, Parravicini C, Muratori S, Tomasini D, Cerri A, Schiro R, Cattorietti G, Delia D, Vago L: Workshop anti-myeloid antibodies: an APAAP immunoenzymatic study of normal tonsil, lymph node, thymus, spleen, bone marrow, liver, lung and skin. In: Mc Michael AJ (ed.). Leukocyte Typing III. Oxford University Press, Oxford, New York, Tokyo, 1987, pp 678–681
32. Hood AF, Vogelsang GB, Black LP, Farmer ER, Santos GW: Acute graft versus host disease. Development following autologous and syngeneic bone marrow transplantation. Arch Dermatol 123:745–750, 1987
33. Sentman CL, Hackett J Jr, Kumar V, Bennet M: Identification of a subset of murine natural killer cells that mediates rejection of Hh-1d but not Hh-1b bone marrow grafts. J Exp Med 170:191–202, 1989
34. Bordignon C, Daley JP, Nakamura I: Hematopoietic histoincompatibility reactions by NK cells in vitro: model for genetic resistance to marrow grafts. Science 230:1398–1401, 1985
35. Ciccone E, Viale O, Bottino C, Pende D, Mignone N, Casorati G, Tambussi G, Moretta A, Moretta L: Antigen recognition by human T cell receptor gamma positive lymphocytes. Specific lysis of allogeneic cells after activation in mixed lymphocyte culture. J Exp Med 167:1517–1522, 1988
36. Reittie JE, Gottlieb D, Heslop HE, Leger O, Drexler HG, Hazlehurst G, Hoffbrand AV, Prentice HG, Brenner MK: Endogeneously generated activated killer cells circulate after autologous and allogeneic marrow transplantation but not after chemotherapy. Blood 73:1351–1358, 1989
37. Mowat AM, Borland A, Parrott DM: Augmentation of natural killer cell activity by anti-host delayed-type hypersensitivity during the graft-versus-host reaction in mice. Scan J Immunol 22:389–399, 1985
38. Ghayur T, Seemayer TA, Lapp WS: Prevention of murine graft-versus-host disease by inducing and eliminating ASGM1+ cells of donor origin. Transplantation 45:586–590, 1988
39. Charley MR, Mikhael A, Hackett J, Kumar V, Bennet M: Mechanism of anti-asialo GM1 prevention of graft-versus-host disease: identification of allo-antigen activated T cells. J Invest Dermatol 91:202–206, 1988
40. Ghayur T, Seemayer TA, Kongshavn PA, Gartner JG, Lapp WS: Graft-versus-host reactions in the beige mouse. An investigation of the role of host and donor natural killer cells in the pathogenesis of graft-versus-host disease. Transplantation 44:261–267, 1987
41. Guy-Grand D, Vassalli P: Gut injury in mouse graft-versus-host reaction: study of its ocurrence and mechanisms. J Clin Invest 77:1584–1591, 1986
42. Piguet PF, Grau GE, Allet B, Vassalli P: Tumor necrosis factor-cachectin is an effector of skin and gut lesions of the acute phase of graft-versus-host disease. J Exp Med 166:1280–1289, 1987
43. Sale GE, Galluci BB, Schubert MM, Sullivan KM, Thomas ED: Direct ultrastructural evidence of target-directed polarization by cytotoxic lymphocytes in lesions of human graft-vs-host disease. Arch Pathol Lab Med 111:333–336, 1987
44. Gale RP: Graft-versus-host disease. Immunol Rev 88:195–214, 1985
45. Hervé P, Wijdenes J, Bergerat JP, Bordigoni P, Milpied N, Cahn JY, Clement C, Beliard R, Morel-Fourrier B, Racadot E, Troussard X, Benz-Lemoine E, Gaud C, Legros M, Attal M, Kloft M, Peters A: Treatment of corticosteroid resistant acute graft-versus-host disease by in-vivo administration of anti-interleukin-2 receptor monoclonal antibody (B-B10). Blood 75:1017–1023, 1990