Transplant Immunology 1996, **4** 151–157

Functional expression of minor histocompatibility antigens on human peripheral blood dendritic cells and epidermal Langerhans cells

Ellen van Lochem[a], Maarten van der Keur[b], A Mieke Mommaas[c], Gijsbert C de Gast[d] and Els Goulmy[a]

[a] *Department of Immunohematology and Bloodbank,* [b] *Department of Hematology and* [c] *Department of Dermatology, Leiden University Hospital,* [d] *Department of Hematology, Utrecht University Hospital*

Received 25 September 1995 accepted for publication 25 October 1995

Abstract. Adequate presentation and cell surface expression of foreign minor histocompatibility antigens (mHag) to allogeneic T cells can lead to graft versus host disease (GvHD) after HLA matched bone marrow transplantation (BMT) Cells of the dendritic cell (DC) lineage including epidermal Langerhans cells (LC) are the most potent inducers of primary alloreactive T cell responses *in vivo* and *in vitro* To explore the possible role of peripheral blood DC and of skin derived LC in the induction of alloimmune responses against mHag we analysed the functional expression of mHag on these professional antigen presenting cells (APC) To this end cytotoxic T cell (CTL) clones specific for mHag H Y and HA 1 to HA 4 were used to demonstrate the presence of these antigens on highly purified DC and LC Our results demonstrate that like other cells of the hematopoietic lineage DC and LC express all the mHag tested for The functional expression of mHag on these potent APC suggests their involvement in the induction of mHag specific GvH directed T cell responses after allogeneic BMT

Introduction

Graft versus host disease (GvHD) is still a major complication of allogeneic bone marrow transplantation (BMT) In HLA matched BMT minor histocompatibility antigen (mHag) disparities between bone marrow donor and recipient can play a role in the development of T cell mediated GvHD [1,2] The alloresponse to mHag *in vivo* is supposed to be initiated by professional antigen-presenting cells (APC)

Cells of the dendritic cell (DC) lineage are well characterized professional APC They are highly efficient in the initiation of primary *in vitro* T cell responses such as in allogeneic mixed leucocyte reactions [3] Moreover DC pulsed with protein antigen *in vitro* or virally infected DC are capable of priming antigen specific MHC restricted T cells both *in vitro* and *in vivo* [4–6] DC are derived from bone marrow precursors and migrate in an immature form via the blood to the non-lymphoid tissues such as the skin [7–9] Epidermal Langerhans cells (LC) are the best characterized nonlymphoid or interstitial DC They are referred to as the peripheral outpost of the immune system, with a special role in the local cutaneous defence [10,11] Within normal epidermis, LC are the only cells that express MHC class II molecules and have the capacity to initiate antigen specific T cell responses [10,12] Like lymphoid DC, LC are efficient APC in the induction of T cell responses [13,14] Following acquisition and processing of antigens, the LC migrate as veiled cells through the afferent lymphatics into the draining lymph nodes where they become specialized at clustering and activating naive T cells [12,15,16] A role for LC in the induction of GvHD has been postulated as the skin is one of the main target organs in GvHD Host LC can persist for a long time after BMT before the epidermis is

Address for correspondence E Goulmy Department of Immunohematology and Bloodbank Leiden University Hospital Bldg 1 E3 Q POB 9600 2300 RC Leiden The Netherlands

repopulated by LC from donor origin [8,17,18] Furthermore, LC are the critical stimulator cells in the *in vitro* allogeneic epidermal cell–lymphocyte reaction, which may be regarded as an *in vitro* correlate of the GvH reaction [19,20]

Objective

The objective of the present study was to investigate the possible role of peripheral blood DC (as possible precursors of LC) and LC as potent inducers of the mHag specific GvH directed T cell responses, by evaluating the functional expression of mHag on these APC mHag generally fail to be recognized by antibodies We therefore used well-characterized mHag specific CTL clones as effector cells and assayed them against highly purified DC and LC as target cells in a cytotoxicity assay

Material and methods

mHag and MHC class I specific cytotoxic T cell clones

Cytotoxic T cell (CTL) clones specific for the mHag H Y and HA-1, HA 2, HA-3 and HA 4 were characterized previously and are described in detail elsewhere [1,21] The CTL clones defining mHag H Y, HA 1, HA-2 and HA 4 are HLA-A2 restricted, whereas mHag HA 3 is recognized in the context of HLA A1 Therefore, MHC class I reactive CTL clones specific for the restriction molecules HLA A2 and HLA A1 were used as control effector cells in the cytotoxicity assays The characteristics of the mHag and MHC class I specific CTL clones are summarized in Table 1 The CTL clones were thawed and cultured for 2 days on rIL 2 (recombinant interleukin 2, 20 U/ml) before being used as effector cells in a ^{51}Cr-release assay

Isolation of peripheral blood DC

Buffy coats derived from 0 5 l of blood from six healthy HLA and mH antigen typed blood donors were used as source for peripheral blood mononuclear cells (PBMC) Enrichment for peripheral blood DC from PBMC was performed according to the method described by Freudenthal and Steinman [22] As a final step in the dendritic cell enrichment procedure, cells were sorted on a flow cytometer (FACStar, Becton Dickinson) Cells in the metrizamide low-density fraction were labeled with a mix of phycoerythrin (PE) conjugated mAb (monoclonal antibody) specific for CD3, CD14, CD16, CD20 and CD56 and fluorescein (FITC) conjugated mAb specific for HLA DR (all from Becton Dickinson, Belgium) Cells negative for the lineage specific markers and highly positive for HLA-DR were sorted (Figure 1a) The sorted cell fraction was analysed for ultrastructure by electron microscopy, and reanalysed on a FACScan (Becton Dickinson) to confirm its purity Expression of high levels of HLA DP and HLA DQ in addition to the absence of lineage specific markers (other than used for sorting, i e CD2, CD33, CD57 and CD19) was used to stain the sorted DC

Isolation of Langerhans cells from epidermal cell suspensions

Epidermal cell suspensions were obtained from skin of five female patients undergoing reconstructive plastic surgery of the breast or abdomen and were prepared as described by Stingl *et al* [23] The patients could not be prospectively typed for HLA and mH antigens The epidermal cell suspensions were stained for CD1a (OKT6 from Ortho Diagnostics) and processed on a FACStar Cells which were highly positive for CD1a were sorted (Figure 1b) The sorted cell fraction was reanalysed on a FACScan and for ultrastructure by electron microscopy Ultrathin cryosections were incubated with anti HLA class II mAb (PdV5 2) conjugated to 10 nm colloidal gold particles [24]

Table 1 Characteristics of mHag and MHC class I specific CTL clones used

CTL clone designation	HLA[b] restriction/specificity	mH antigen		
		Code	Phenotype frequency (%)	Tissue[d] distribution
cl 2	A1			
3E2	A2			
3HA15	A2	HA 1	69	Hematopoietic
5H13	A2	HA 2	94	Hematopoietic
5HO11	A1	HA 3	88	Ubiquitous
5G30	A2	HA 4	16	Ubiquitous
1R35	A2	H Y	Male	Ubiquitous

From Goulmy[1] and Van Els *et al* [21]

[b] The CTL clones used in this study recognize mHag in association with either HLA A1 (5HO11) or HLA A2 (3HA15 5H13 5G30 and 1R35)

Phenotype frequencies of the mHag in the HLA A1 (HA 3) or HLA A2 (HA 1 HA 2 HA 4 H Y) positive healthy population

[d] From De Bueger *et al* [30] cell types derived from several tissues were analysed for their mHag expression The tissue distribution of HA 1 and HA 2 was restricted to cells of the hematopoietic lineage (PHA blasts EBV LCL purified T cells B cells monocytes and immature thymocytes), while mHag HA 3 HA 4 and H Y were detected on all tissues tested hematopoietic and nonhematopoietic cells (cultured fibroblasts keratinocytes melanocytes cultured epithelial cells of kidney proximal tubuli and umbilical cord vein derived endothelial cells)

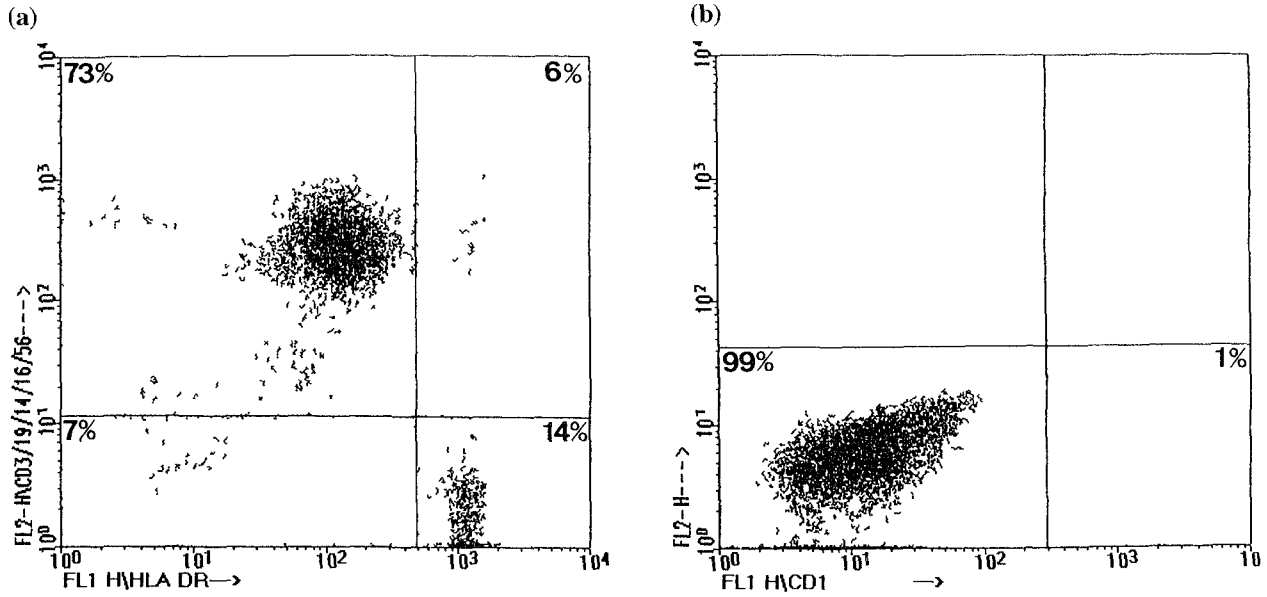

Figure 1 Sorting parameters for the isolation of peripheral blood DC and epidermal LC (a) Expression of high levels of HLA DR (FL 1) in addition to the absence of lineage specific markers (FL 2) were used as sorting parameters (lower right quadrant) to purify the enriched DC fractions (b) Langerhans cells were sorted from epidermal cell suspensions 1 2% of the single cell suspensions stained for the LC specific marker CD1a (FL 1)

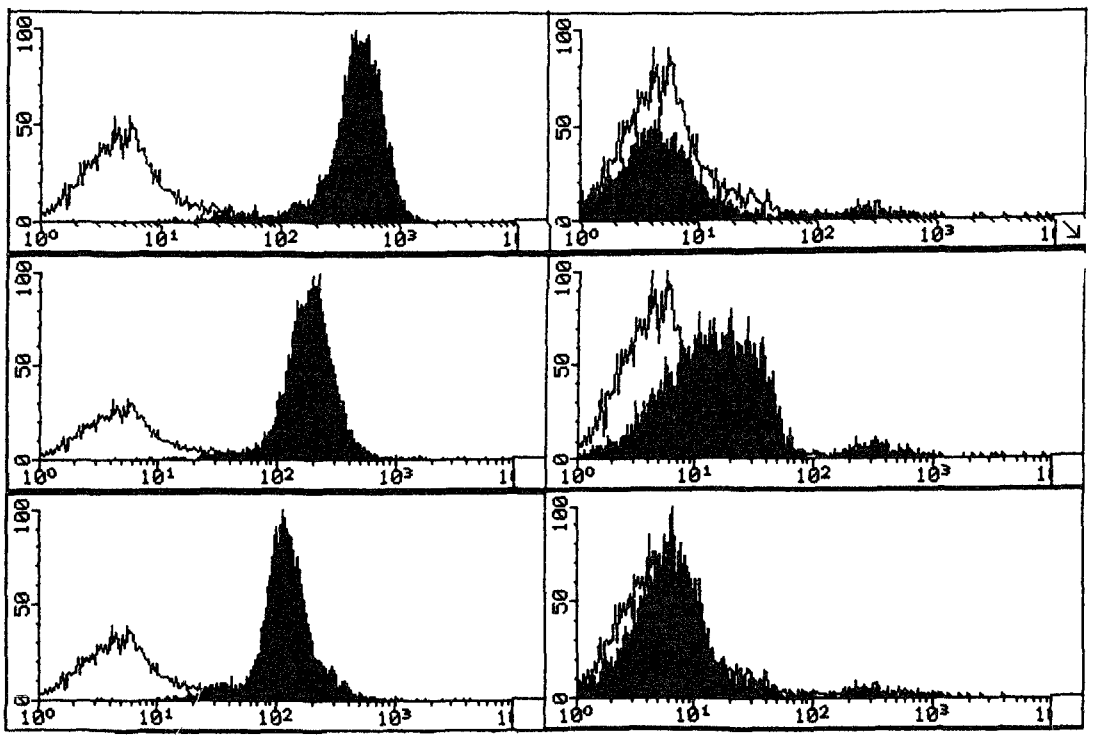

Figure 2 FACS analysis of the sorted peripheral blood DC Sorted DC were stained with mAb specific for HLA DP (B7 21 Becton Dickinson) and HLA DQ (SPvL3 from H Spits) for B7 1 and for the lineage specific markers CD2 CD19 and CD33 followed by PE conjugated goat anti mouse mAb (background peak)

Cytotoxicity assay

Expression of the CTL defined mHag on purified peripheral blood DC and LC was analysed in a standard 4 h ^{51}Cr release assay FACS-sorted DC and LC were used as target cells for the mHag and MHC class I specific CTL clones in effector to target ratios of 20 1 and 2 1 LC were incubated with IFN-γ (interferon gamma) 200 U/ml for 48 h at 37°C before being used as target cells Percentages of specific ^{51}Cr release were calculated as follows

$$\% \text{ lysis} = \frac{\text{experimental release} - \text{spontaneous release}}{\text{maximal release} - \text{spontaneous release}} \times 100$$

in which the spontaneous ^{51}Cr release by the different target cells is always lower than 20% FACS sorted DC, pure fractions of monocytes (> 95% CD14 positive) and phytohemagglutinin (PHA) stimulated T cell blasts from the same blood donor were tested simultaneously as target cells

Results

Purity of the FACS-sorted DC and LC

FACS sorting provided high purity of both DC and LC FACS analysis of the sorted DC and LC revealed that > 95% of the cells expressed relatively high amounts of HLA DP and HLA DQ and were positive for B7 1 analogous to what was described earlier [22 25] the sorted DC were negative for lineage specific markers such as CD2 CD19 and low positive for CD33 (Figure 2) Ultrastructural analysis of the sorted DC (Figure 3) and LC (Figure 4a) demonstrated that these cells both displayed the specific characteristics such as long cytoplasmic processes and a lobulated nucleus the sorted CD1a positive cells clearly displayed Birbeck granules (Figure 4a inset) Immunoelectron microscopy revealed a relatively high expression of class II molecules on the LC, both intracellularly (Figure 4b) and on the cell surface (data not shown) Ultrastructural and FACS analysis of the sorted LC furthermore demonstrated that the few keratinocytes contaminating the fractions were not viable

mHag and HLA expression of peripheral blood DC

Purified preparations of peripheral blood DC from six blood donors were analysed for expression of the mHag H Y and HA 1 HA 2 HA 3 and HA 4 and of the relevant restriction molecules HLA A2 and HLA A1 DC as well as PHA blasts and monocytes of each individual were assayed simultaneously as target cells with mHag and MHC class I specific CTL The specific lysis percentages of the target cells from three of the six donors are depicted in Table 2 It is clear that DC were equally susceptible to lysis by the different CTL clones as the PHA blasts and monocytes of the same donor Thus analogous to PHA blasts and monocytes the mHag HA 1 HA 2 HA 3 HA 4 and H Y are clearly recognized on peripheral blood DC

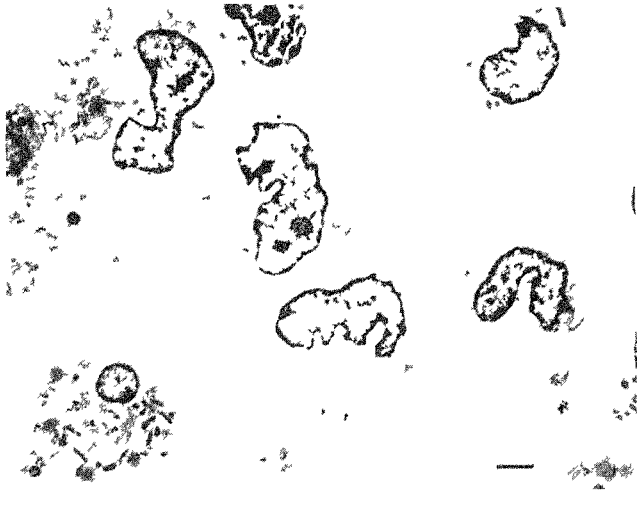

Figure 3 Electron micrograph of the sorted CD3 CD14 CD16 CD20 CD56 negative HLA DR positive cells showing the characteristics morphology of DC such as cytoplasmic processes and a lobulated nucleus Bar = 2 μm

Figure 4 (a) Electron micrographs of sorted CD1 positive cells showing LC with specific characteristics such as long cytoplasmic processes a lobulated nucleus and Birbeck granules (inset bar = 0 5 μm) in the cytoplasm of the sorted CD1 positive cells Bar – 2 μm (b) Incubation of ultrathin cryosections with anti HLA class II mAb (PdV5 2) conjugated to 10 nm colloidal gold particles[24] revealed a relatively high HLA class II expression on intracellular vesicular structures of the sorted LC Birbeck granules were negative Bar – 0 5 μm

mHag and HLA expression of epidermal LC

Pure preparations of LC obtained from five female individuals were analysed for expression of the mHag HA 1 HA 2 and HA 3 and for their relevant restriction molecules HLA A2 and HLA A1 As LC were obtained solely from female individuals expression of the male specific mHag H Y could not be analysed Table 3 shows the specific lysis percentages of the

Table 2 Lysis of peripheral blood DC by mHag and MHC class I specific CTL

mH/MHC[b] specificity		Individuals[a]								
		D-A[c]			D-B			D-C		
	E T[d]	PHA	Mono	DC	PHA	Mono	DC	PHA	Mono	DC
HLA A1	20	83[e]	48	81	6	nt	nt	7	nt	0
	2	75	27	61	3	nt	nt	7	nt	0
HLA A2	20	82	56	83	60	43	77	82	57	61
	2	63	30	52	41	15	55	63	20	60
HA-1	20	71	35	68	79	60	104	50	31	52
	2	62	11	50	75	51	79	40	25	23
HA 2	20	47	30	45	43	34	73	35	36	38
	2	28	12	26	38	24	64	26	18	22
HA-3	20	93	69	85	0	–2	1	3	6	nt
	2	81	38	67	–3	0	4	0	0	nt
HA-4	20	0	–4	0	82	67	93	1	2	2
	2	1	5	0	76	45	88	–1	nt	nt
H-Y	20	83	44	77	–1	nt	nt	57	32	41
	2	76	31	73	0	nt	nt	51	20	40

[a] PHA stimulated T cell blasts (PHA), monocytes (mono) and dendritic cells (DC) are tested simultaneously as target for lysis by mH specific CTL
[b] Antigen specificity of the clones used (see Table 1 for further information)
[c] HLA type of individual D-A (male), A1,2, B7,8, Cw7, DR15,3, DQ1,2, DPw1,w4, individual D-B (female), A2, B27, 40, Cw2,w3, DR11,12, DQ3, DPw4, individual D-C (male), A2,24, B44,62, Cw3,w5, DR13,4, DQ3,6 DPw3
[d] Effector target ratio used in the ^{51}Cr release assay
[e] Percentages of specific lysis in a 4-h ^{51}Cr-release assay

Table 3 Lysis of epidermal Langerhans cells by mHag and MHC class I specific CTL

mH/MHC specificity[c]	E T[b]	Individuals[a]					
		L-A		L-B	L-C	L-D	L-E
		–[d]	+	+	+	+	+
HLA-A1	20 1	21[e]	71	5	0	3	93
	2 1	18	nt	0	0	0	87
HLA-A2	20 1	2	7	48	51	61	74
	2 1	0	nt	nt	42	42	67
HA-1	20 1	3	5	32	57	32	72
	2 1	1	nt	23	38	nt	59
HA-2	20 1	0	3	35	32	76	64
	2 1	0	nt	31	21	43	43
HA-3	20 1	19	83	0	2	5	72
	2 1	12	nt	0	0	1	70

[a] LC were obtained from epidermal cell suspensions from skin of five individuals
[b] Effector target ratio used in the ^{51}Cr-release assay
[c] Antigen specificity of the clones used (see Table 1 for further information)
[d] LC were used as target for mHag specific lysis after preincubation with (+) IFN-γ, LC of individual L-A are tested for lysis susceptibility after preincubation with (+) and without (–) IFN γ
[e] Percentages of specific lysis in a 4 h ^{51}Cr-release assay

LC from the five individuals by the different CTL clones LC of individual L-A were tested as target cells after a 48-h incubation with and without IFN-γ The viability of the LC fraction was maintained during this culture step, in which no other cytokines were added Preliminary FACS analyses of LC of individual L-A, incubated with or withuot IFN-γ, revealed a significant increase in the expression of ICAM-1, B7-1 and MHC molecules on the surface of the LC after IFN-γ incubation (data not shown) Although freshly isolated LC were susceptible to mHag specific lysis by CTL clones (individual L-A$^-$), the lysis susceptibility was markedly enhanced by preincubating the LC for 48 h with IFN-γ 200 U/ml (individual L-A$^+$) Therefore, LC of individuals L-B, L-C, L-D and L-E were preincubated with IFN-γ before being used as target cells The LC are found to functionally express the mHag HA-1, HA-2 and HA-3 (Table 3)

Discussion

The influence of mHag mismatches between bone marrow donor and recipient on the development of GvHD after HLA genotypically identical BMT[1] was recently confirmed in a prospective multicentre study Mismatches for mHag HA-1, -2, -4, or -5 was observed to be significantly associated with the occurrence of GvHD [26] The induction of T cell responses to mHag mismatches will depend largely on the type of APC by which the antigens are presented Namely, when the T cell receptor/MHC interaction is not aided by appropriate costimulatory signals, the T cell can be anergized and will be unresponsive to subsequent triggering by professional APC Non-

professional APC such as keratinocytes and fibroblasts have been found to induce T cell tolerance to the mHag they express [27] Therefore, the induction of mHag specific T cell responses after BMT is supposed to be controlled by professional APC, expressing high levels of costimulatory molecules Cells of the DC lineage, including interstitial DC such as LC, are described to be the most potent APC in the induction of primary T cell responses both *in vitro* and *in vivo* [6 10 11] The high levels of MHC molecules and the expression of costimulatory molecule B7-1 on the surface of these professional APC after activation[22 28 29] (Figure 2) contribute to their superior antigen-presenting capacity

The expression of mHag on peripheral blood DC and LC, as described in the present study, could indicate a role for cells of the DC lineage in the initiation of mHag specific T cell responses in GvHD Although the recipient is depleted for most hematopoietic derived cells prior to the BMT, residual host leucocytes including cells of the DC lineage are still present at the moment of BMT In the skin, residual host LC can persist for a long time after BMT before the host epidermis is repopulated by LC of donor origin [17]

On the one hand, with their superior antigen-presenting capacity, these residual host mHag expressing cells of the DC lineage might be involved in the primary induction of GvH directed mHag specific T cell responses after BMT Host LC migrating from the skin through the afferent lymph into the draining lymph nodes, upregulating the expression of MHC and accessory molecules, could stimulate the naive donor derived T cells they encounter [2 4–6] Once sensitized by the mHag expressing DC or LC, the activated T cells could readily interact with other mHag positive cells, such as keratinocytes in the skin [30]

On the other hand, the residual host DC and LC may function as target cells for, or restimulate already sensitized, mHag specific T cells in GvHD T cells of donor origin may be primed by residual host leucocytes in the circulation, and reactivated by the most potent APC in the skin, the LC Decreased numbers of LC in the skin during the course of cutaneous GvHD, as was shown in immunohistochemical studies, suggest a role of LC as target cells [18 31] In that case, the damage to keratinocytes may be a nonspecific effect of lymphokine release during the lymphocyte/LC interactions Studies on murine LC indicate low or absence of expression of at least some MHC class I on the surface of LC, making them less susceptible to alloreactive cytotoxic T cell attack [32] In our present study, we found that the low lysis susceptibility of freshly isolated human epidermal LC by MHC class I and mHag specific CTL clones was upregulated after incubation with IFN-γ Preliminary FACS analyses revealed that IFN-γ may enhance CTL recognition by the upregulation of MHC molecules, B7 1 and adhesion molecules such as ICAM-1 As there were no other cytokines added to the culture and no viable cytokine-producing cells such as keratinocytes could be detected in the sorted fraction, we ascribe this effect solely to IFN γ The *in situ* release of IFN-γ during cutaneous GvHD[33] may thus effectively enhance the recognition of LC by mHag specific CTL clones after BMT, although the data presented in this study cannot directly be extrapolated to an *in vivo* role of the mHag specific CTL in the GvHD pathogenesis Our study on the functional recognition of mHag on LC and DC links up with previous studies on the tissue distribution of mHag [30] The results of this study demonstrate that, like other cells of the hematopoietic lineage, DC and LC express all the mHag we tested for (i e H Y and HA-1 to HA-4 on the peripheral blood DC, and HA-1, HA 2 and HA-3 on the epidermal LC) The expression of mHag on LC and DC strongly suggests a possible involvement of cells of the DC lineage in GvH directed mHag specific T cell responses after allogeneic BMT

Acknowledgements

The authors would like to thank M Hurks and C Out for making the epidermal cell suspensions, A van der Marel for help with the FACS sorter, A Mulder and I Schadee for making the electron micrographs and F Claas for critically reading the manuscript This work was supported by grants from the Dutch Cancer Foundation (Koningin Wilhelmina Fonds) and the JA Cohen Institute for radiopathology and radiation protection

References

1 Goulmy E Minor histocompatibility antigens and their role in transplantation *Transplant Rev* 1988 **2** 29–53
2 Korngold R, Sprent J Lethal GvHD across minor histocompatibility barriers nature of the effector cells and role of the H 2 complex *Immunol Rev* 1983, **71** 5–29
3 Steinman RM, Witmer MD Lymphoid dendritic cells are potent stimulators of the primary mixed leukocyte reaction in mice *Proc Natl Acad Sci USA* 1978, **75** 5132
4 Macatonia SE, Taylor PM, Knight SC, Askonas BA Primary stimulation by dendritic cells induces antiviral proliferative and cytotoxic T cell responses *in vitro* *J Exp Med* 1989, **169** 1255 64
5 Inaba K Metlay JP Crowley MT, Steinman RM Dendritic cells pulsed with protein antigens *in vitro* can prime antigen-specific, MHC restricted T cells *in situ* *J Exp Med* 1990, **172** 631–40
6 Mellief CJM Dendritic cells as specialized antigen presenting cells *Res Immunol* 1989, **140** 902–906
7 Katz SI, Tamaki K, Sachs DH Epidermal Langerhans cells are derived from cells originating in the bone marrow *Nature* 1979, **282** 324–26
8 Pelletier M, Perrault C, Landry D, David M, Montplaisir S Ontogeny of human epidermal Langerhans cells *Transplantation* 1984, **38** 544–46
9 Romani N Schuler G Structural and functional relationships between epidermal Langerhans cells and dendritic cells *Res Immunol* 1989 **140** 895–98
10 Streilein JW, Bergstresser PR Langerhans cells antigen presenting cells of epidermis *Immunobiology* 1984, **168** 285–300
11 Stingl G Tamaki K Katz SI Origin and function of epidermal Langerhans cells *Immunol Rev* 1980 **53** 149–74
12 Romani N Schuler G The immunologic properties of epidermal Langerhans' cells as a part of the dendritic cell system *Immunopathology* 1992, **13** 265–79
13 Braathen LR, Thorsby E Human epidermal Langerhans cells are more potent than blood monocytes in inducing some antigen-specific T cell responses *Br J Dermatol* 1983, **108** 139–46
14 Moulon C, Peguet Navarro J, Courtellemont P Redziniak G Schmitt D *In vitro* primary sensitization and restimulation of hapten-specific T cells by fresh and cultured human epidermal Langerhans' cells *Immunology* 1993, **80** 373–79
15 Hoefsmit ECM, Duijvestijn AM, Kamperdijk WA Relation between Langerhans cells, veiled cells, and interdigitating cells *Immunobiology* 1982, **161** 255–65
16 Kripke ML, Munn CG, Jeevan A Tang J Bucana C Evidence that cutaneous antigen presenting cells migrate to regional lymph nodes during contact sensitization *J Immunol* 1990, **145** 2833–37
17 Perrault C Pelletier M Belanger R *et al* Persistence of host Langerhans cells following allogeneic bone marrow transplantation possible relationship with acute graft-versus host disease *Br J Haematol* 1984, **60** 253–60

18 Breatnach SM, Katz SI Immunopathology of cutaneous graft-versus-host disease *Am J Dermatopathol* 1987, **9** 343–48
19 Faure M, Flappaz A, Schmitt D, Dezutter-Dambuyant C, Thivolet J Role of HLA DR bearing Langerhans and epidermal indeterminate cells in the *in vitro* generation of alloreactive cytotoxic T cells in man *Cell Immunol* 1984, **83** 271–79
20 Bagot M, Mary JY, Heslan M *et al* The mixed epidermal cell lymphocyte reaction is the most predictive factor of acute graft versus host disease in bone marrow graft recipients *Br J Haematol* 1988, **70** 403–409
21 Van Els CACM, D'Amaro J, Pool J *et al* Immunogenetics of human minor histocompatibility antigens their polymorphism and immunodominance *Immunogenetics* 1992, **35** 161–65
22 Freudenthal PS, Steinman RM The distinct surface of human blood dendritic cells, as observed after an improved isolation method *Proc Natl Acad Sci USA* 1990, **87** 7698–702
23 Stingl G, Gazze-Stingle LA, Aberer W, Wolff K Antigen presentation by murine epidermal Langerhans cells and its alteration by ultraviolet B light *J Immunol* 1981, **127** 1707–13
24 Mommaas AM, Wijsman MC, Mulder AA, van Praag MCG, Vermeer BJ, Koning F HLA class II expression on human epidermal Langerhans cells *in situ* upregulation during allergic contact dermatitis *Hum Immunol* 1992, **34** 99–106
25 Egner W, McKenzie JL, Smith SM, Beard MEJ, Hart DNJ Identification of potent mixed leukocyte reaction-stimulatory cells in human bone marrow, putative differentiation stage of human blood dendritic cells *J Immunol* 1993, **150** 3043–53
26 Goulmy E *et al N Engl J Med* (in press)
27 De Bueger M, Bakker A, Goulmy E Acquired tolerance for minor histocompatibility antigens after HLA identical bone marrow transplantation *Int Immunol* 1992, **4** 53–57
28 Young JW, Koulova L, Soergel SA, Clark EA, Steinman RM, Dupont B The B7/BB1 antigen provides one of several costimulatory signals for the activation of CD4$^+$ T lymphocytes by human blood dendritic cells *in vitro* *J Clin Invest* 1992, **90** 229–37
29 Symington FW, Brady W, Linsley PS Expression and function of B7 on human epidermal Langerhans cells *J Immunol* 1993, **150** 1286–95
30 De Bueger M, Bakker A, van Rood JJ, van der Woude F, Goulmy E Tissue distribution of human minor histocompatibility antigens Ubiquitous versus restricted tissue distribution indicates heterogeneity among human CTL defined non-MHC antigens *J Immunol* 1992, **149** 1788–94
31 Breatnach SM, Shimada S, Kovac Z, Katz S Immunologic aspects of acute cutaneous graft-versus-host disease decreased density and antigen-presenting function of Ia$^+$ Langerhans cells and absent antigen-presenting capacity of Ia$^+$ keratinocytes *J Invest Dermatol* 1986, **86** 226–34
32 Sharrow SO, Barden LA, Singer A, Katz SI Quantitative differences in cell surface expression of class I MHC antigens on murine epidermal Langerhans cells *J Immunol* 1994, **153** 110–16
33 Ferrara JL Cytokine dysregulation as a mechanism of graft versus host disease *Curr Opin Immunol* 1993, **5** 794–99