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Expression of functional molecules by human CD3$^-$ decidual granular leucocyte clones

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SUMMARY

Cell surface and cytoplasmic antigen expression by 35 CD3$^-$ decidual granular leucocyte (DGL) clones, derived from human endometrial tissue in the first trimester of pregnancy, has been compared with both that of fresh CD3$^-$ decidual leucocytes and that of CD3$^-$ peripheral blood natural killer (PBNK) cell clones ($n = 12$). The majority of DGL clones retained the antigenic phenotype of fresh cells, although CD103 (HML-1) was expressed on 50% of DGL clones but only 17% of fresh DGL. Both cytoplasmic CD3ζ and CD3ε chains were detected in $>90\%$ of DGL clones in the absence of cell surface CD3. Cytoplasmic CD3ζ was present in almost all fresh CD3$^-$ DGL, whereas CD3ε was not. Most DGL clones did not express surface Fcγ receptors I-III (CD64, -32 and -16, respectively) and complement receptors (CR) types 1 and 2 (CD35 and 21, respectively), but 43% expressed CR3 (CD11b/18); in contrast, all PBNK clones were CR3$^+$. The NK cell-associated molecules Kp43 (CD94) and the p58 molecule recognized by the HP3E4 monoclonal antibody were both present on a higher proportion of CD3$^-$ PBNK (91% and 50%, respectively) than DGL clones (31% and 14%, respectively), despite expression of CD94 by $>90\%$ of fresh CD56$^+$ decidual leucocytes. Five of 35 CD3$^-$ DGL clones expressed cytoplasmic CD3ζ in the absence of expression of CD2, CD16 or the p58 molecule recognized by HP3E4. These variations between CD3$^-$ DGL and PBNK cell clones in expression of functional molecules may be related to previously reported differences in major histocompatibility complex-non-restricted cytotoxic activities between these two cell types.

INTRODUCTION

Human decidual granular leucocytes (DGL) mostly have the cell surface antigenic phenotype CD2$^+$ CD3$^-$ CD4$^-$ CD7$^+$ CD8$^-$ CD16$^-$ CD56^{bright} CD57$^-$,[1] similar to a small subset of peripheral blood large granular lymphocytes.[2] These cells are pronounced in the late secretory phase of the non-pregnant endometrium and accumulate further in the decidualized endometrium during the first trimester of pregnancy, but apparently decline thereafter.[1] CD3$^-$ DGL can lyse natural killer (NK)-sensitive target cell lines but, although they possess cytolytic molecules such as perforin and serine esterases,[3] they are poor major histocompatibility complex (MHC)-non-restricted cytolytic effectors compared with peripheral blood NK (PBNK) cells.[4] In addition, DGL are able to suppress the mixed lymphocyte culture reaction[5,6] and show decreased lysis of HLA-G$^+$ compared with control HLA-G$^-$ target cells.[5,7]

Three distinct cell surface complement receptors have been identified: CR1 (CD35), CR2 (CD21) and CR3 (CD11b/CD18).[8] CR1 and CR3 facilitate phagocytosis of complement-coated bacteria and CR1 on erythrocytes also plays a role in clearing immune complexes from the circulation. CR1 binds C3b/C4b and is also expressed by B cells, macrophages and neutrophils; CR2 binds C3d and is expressed mainly by B cells, and CR3 binds C3bi and is expressed by macrophages, neutrophils and PBNK cells. There are also three different types of leucocyte Fcγ receptors,[9] FcγRI (CD64) is a high-affinity receptor on monocytes and macrophages that can be induced on neutrophils by interferon-γ. Three structural forms of FcγRII (CD32) have been described. Two forms are expressed on neutrophils, while a third occurs on lymphocytes; all forms are found on monocytes. The low-affinity FcγRIII (CD16) is expressed on the surface of neutrophils in a glycosyl phosphatidylinositol-anchored form while, on NK cells and macrophages, it occurs as a transmembrane protein.[9]

Human PBNK cells and a subset of CD3$^+$ $\gamma\delta$ T cells express Kp43 (CD94), a type II membrane protein with a C-type lectin domain,[10] that has been associated with MHC-non-restricted cytotoxic activity.[11] The p58 molecule identified by the murine monoclonal antibody (mAb) HP3E4, is expressed by a subset of peripheral NK cells.[12] Both of these molecules are thought to interact with products of specific class I human leucocyte

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antigen (HLA) alleles on target cells, resulting in inhibition of NK cell-mediated cytolytic activity.[13,14]

The CD3ζ molecule occurs as a transmembrane homo- or hetero-dimer (ζ–γ) in T cells and NK cells, where it has a predominantly cytoplasmic location. It can be found in association with the T-cell antigen receptor/CD3 complex in T lymphocytes or with the transmembrane form of FcγRIII (CD16) on PBNK cells,[15] and also can occur in association with CD2[16] and the p58 molecules recognized by the GL183 and EB6 mAb.[17] PBNK cells may also express cytoplasmic CD3ε following activation in the absence of surface CD3 expression.[18]

In the present study, we have investigated the expression of a set of functional molecules by $CD3^-$ DGL clones in order to determine whether they differ significantly from PBNK cell clones. Any differences in expression of such molecules between $CD3^-$ PBNK and DGL clones might be related to differences in functional activities between these two cell types.

MATERIALS AND METHODS

Leucocyte isolation

Decidualized endometrial tissue was obtained fresh from elective first-trimester vaginal terminations of normal pregnancy and decidual cells were isolated as described previously.[5,19] Briefly, non-decidual tissue was removed and, after washing several times in phosphate-buffered saline (PBS), pH 7·4, decidua was minced thoroughly between two scalpels and digested for 30 min at room temperature with gentle agitation in RPMI-1640 with 0·1% collagenase (type IV; Sigma, Poole, UK). Extracted cells were then filtered through sterile fine gauze, washed in PBS and centrifuged over Lymphoprep® (Nycomed, Oslo, Norway). Cells accumulating at the interface were collected, washed twice in PBS and labelled for 60 min at 4° with murine anti-CD3 mAb (UCHT1; 1 : 100; Dakopatts, Copenhagen, Denmark), then washed again in ice-cold PBS. These cells were subsequently incubated with magnetic beads coupled to sheep anti-mouse immunoglobulin antiserum (4×10^7 beads/10^6 cells; Dynal, Wirral, UK) for 30 min at 4°; cells bound to the beads were removed using a magnet. Unbound cells were counted prior to use. Cell viability was >90% as determined by trypan blue exclusion.

Peripheral blood mononuclear cells (PBMC) were isolated by centrifugation over Lymphoprep®. Cells accumulating at the interface were washed twice in PBS, pH 7·4, prior to use.

Culture and cloning of $CD3^-$ leucocytes

$CD3^-$ decidual and PBNK cell clones were generated as previously described.[5,19] Briefly, T cell-depleted populations were cloned by limiting dilution in 96-well round-bottomed plates in a feeder mixture containing RPMI-1640, antibiotics, 10% heat- inactivated fetal calf serum (FCS), 2×10^5 allogeneic PBMC/ml, 10^5 B lymphoblastoid cells/ml (both given 5000 rads γ-irradiation), 1 μg/ml indomethacin (Sigma), 100 IU/ml human lymphoblastoid interleukin-2 (IL-2) (Biotest, Dreieich, Germany) and 1 μg/ml phytohaemagglutinin (PHA, Wellcome, Beckenham, Kent). Decidual cells were plated at 5–20 viable cells/well, whereas PBMC were plated at 0·5–2 viable cells/well, and cultured for up to 3 weeks at 37° in an atmosphere of 5% CO_2. Proliferative frequencies were 5–10% for PBMC and 0·5–1·0% for decidual cells. Clones were fed weekly with the same feeder mixture but without PHA, and had a lifespan of 4–6 weeks.[5,19]

Table 1. Murine monoclonal antibodies (mAb) used in this study

mAb	Specificity	Dilution of stock	Source
DAKO-T11	CD2	1:50	Dakopatts
UCHT1	CD3ε	1:50	Dakopatts
DAKO-T8	CD8	1:50	Dakopatts
DAKO-MLA	HML-1; CD103	1:20	Dakopatts
DAKO-C3bR	CR1; CD35	1:20	Dakopatts
DAKO-CD21	CR2; CD21	1:20	Dakopatts
DAKO-C3bi-R	CR3; CD11b/18	1:20	Dakopatts
10.1	FcγRI; CD64	1:20	Serotec*
AT10	FcγRII; CD32	1:20	Serotec
Leu 11b	FCγRIII; CD16	1:20	Becton Dickinson
Leu 19	CD56	1:20	Becton Dickinson
TCR1	pan-αβ TCR	1:20	Becton Dickinson
TIA-2	CD3ζ	1:10	Coulter
Kp43	CD94	1:10	Ref. 11
HP3E4	p58 molecule	1:10	Ref. 13

* Serotec (Oxford, UK).

Flow cytometry

Aliquots of decidual and PBNK clones (10^5 cells) were washed and labelled with a panel of appropriately diluted murine primary mAb (Table 1) for 30 min at 4°. After washing in PBS, pH 7·4, cells were labelled with fluorescein-conjugated goat anti-mouse immunoglobulin antiserum (1 : 50; Sigma) for 30 min at 4° and washed prior to analysis using an EPICS XL flow cytometer (Coulter, Luton, UK). With fresh decidual populations, cells were gated for lymphocytes on the basis of forward and 90° scatter, and results expressed as % staining of the total gated population. For cloned cell populations, viable cells were gated and clones scored as positive if >50% of cells stained with a particular mAb.

For cytoplasmic CD3ε and CD3ζ staining, cells were initially fixed with 2% paraformaldehyde for 10 min at 4°. After washing with PBS, pH 7·4, they were then made permeable with 0·1% saponin (Sigma) in PBS for 20 min. All further steps, incubation with primary mAb and secondary antibodies (fluorescein-conjugated $F(ab')_2$ fragments of sheep–anti-mouse immunoglobulin; Sigma) as well as all washes, were performed in PBS containing 0·1% saponin. In some experiments, coexpression of cytoplasmic antigens (CD3ζ or CD3ε) or cell surface Kp43 with surface CD56 was studied using phycoerythrin-conjugated anti-CD56 mAb (Becton Dickinson, Mountain View, CA). Prior to surface staining, permeabilized cells were allowed to recover for 30 min in ice-cold PBS containing 10% FCS. This method was not employed with DGL clones because of the high endogenous fluorescence of these cells.

Immunocytochemical staining

Fresh or cultured cells, or leucocyte clones, were adjusted to a concentration of 2×10^5 cells/ml. Cytospin smears were made with 100 μl of cell suspension (Cytospin 2; Shandon, Runcorn, UK), air-dried, fixed in acetone for 10 min at room temperature and stored sealed at −70° for subsequent immunocytochemical staining.

Cell smears were first incubated with 10% non-immune goat serum for 20 min. Each sample was incubated with

appropriately diluted primary mAb for 60 min at room temperature and then washed with Tris-buffered saline (TBS), pH 8·0, for 10 min. This was followed by incubation with a secondary goat or rabbit biotinylated anti-mouse immunoglobulin antibody (Zymed, San Francisco, CA) for 45 min, and a wash in TBS for 10 min. Samples were further incubated with extrAvidin® alkaline phosphatase (1 : 1000 dilution; Sigma) for 30 min. After a TBS wash, a substrate–chromogen mixture comprising 2 mg naphthol AS-MX phosphate (Sigma) dissolved in 200 μl dimethylformamide and 10 ml 0·1 M Tris–HCl (pH 8·0) was prepared into which 1 M levamisole was added. Fast red TR salt (Sigma) was then dissolved in this mixture, which was filtered through Whatman 0·2 μm filter paper immediately prior to application to the smears. These were incubated for 10 min and then rinsed with distilled water. Finally, smears were counterstained with haemalum and mounted in aqueous mountant prior to microscopic examination. Samples incubated with the secondary biotinylated anti-mouse immunoglobulin antibody alone were used as negative controls.

RESULTS

Cell surface antigenic phenotype of CD3⁻ DGL and PBNK clones

Thirty-five CD3⁻ DGL clones were derived from seven different fresh first-trimester pregnancy decidual tissue samples and compared with twelve CD3⁻ clones derived from peripheral blood in the same manner. The cloning frequency of CD3⁻ cells from decidual tissues was low (< 1%). The majority of clones (60%) coexpressed CD2 and CD56 antigens (Fig. 1), 34% were positive for either CD2 or CD56, and two clones (6%) expressed neither of these antigens (Table 2). Lack of expression of CD2 and/or CD56 has previously been noted in a minority of CD3⁻ clones from decidual tissue and peripheral blood.[5,19]

The clones displayed marked heterogeneity of expression of antigens tested in this study. Both DGL (n = 35) and PBNK clones (n = 12) were tested for expression of Fcγ receptors (CD16, CD32, CD64), complement receptors (CD11b, CD21, CD35), CD2, CD56, CD8 and CD103, as well as for the expression of the NK cell-associated functional molecules CD94 (Kp43) and the molecule recognized by the HP3E4 mAb (Fig. 1).

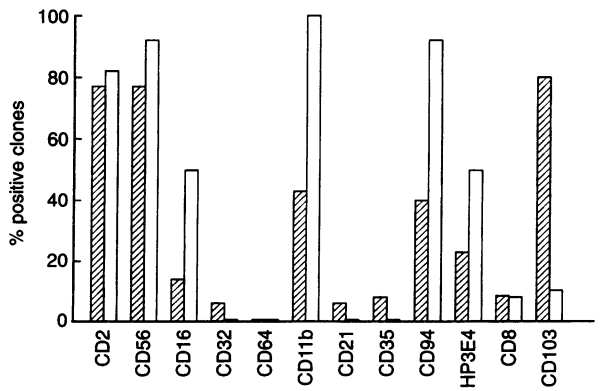

Figure 1. Cell surface antigen expression by CD3⁻ DGL clones (hatched bars; n = 35) and PBNK clones (open bars; n = 12).

Complement and Fcγ receptor expression by CD3⁻ DGL and PBNK clones

CR3 was expressed by 43% of CD3⁻ DGL clones, representing a pronounced difference from CD3⁻ PBNK clones where CR3 was expressed by all clones (Fig. 1). Two (5·7%) DGL clones expressed both CR1 and CR2, and another clone expressed CR1 alone; all three clones also coexpressed CR3 (Table 2). These DGL clones demonstrated only low levels of expression of CR1 and CR2, and these molecules were absent from all PBNK clones (Fig. 1).

Fifty per cent of CD3⁻ PBNK clones, and only 14% of CD3⁻ DGL clones, were positive for CD16 (FcγRIII). CD32 (FcγRII) was weakly expressed by three DGL clones, but was absent from all PBNK clones; only a single DGL clone showed weak expression of CD64 (FcγRI).

Complement and Fcγ receptor expression by fresh decidual cells

Freshly isolated decidual cells were analysed by flow cytometry for expression of complement and Fcγ receptors. CD11b and, to a lesser extent CD16, were the only receptors expressed by a substantial population of cells (Fig. 2); CR1 and -2, and FcγRI and -II, were present on < 10% of cells. As only 40% of decidual cells were $CD56^+$ or $CD2^+$, these populations evidently contained around 50% non-leucocytic cells.

Expression of NK cell-associated molecules

The expression of NK cell-associated molecules was confined entirely to clones that coexpressed CD56. Kp43 (CD94) was detected on 31% of DGL clones (Table 3), but was present on 92% of CD3⁻ PBNK clones (Fig. 1). The p58 molecule recognized by the HP3E4 mAb[13] was expressed by only 14% of DGL clones, but by 50% of PBNK clones (Fig. 1); in most cases, the positive clones coexpressed Kp43, but three DGL clones were positive only with HP3E4 (Table 2). These relative proportions were reflected also in fresh CD3⁻ decidual leucocyte populations (Fig. 2).

Freshly isolated decidual cells were double labelled with anti-CD56 and anti-Kp43, and analysed by flow cytometry. All $Kp43^+$ cells coexpressed CD56, but < 10% of $CD56^+$ cells were $Kp43^-$ (data not shown).

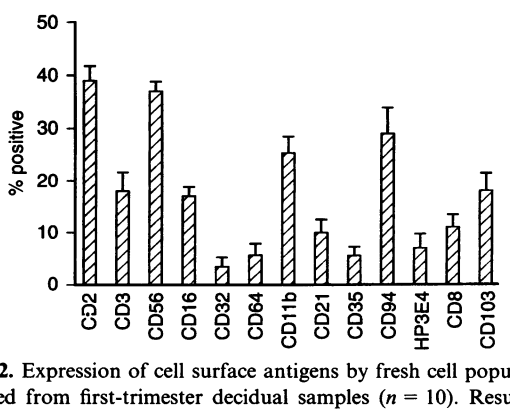

Figure 2. Expression of cell surface antigens by fresh cell populations extracted from first-trimester decidual samples (n = 10). Results are shown as mean % positive cells ± SEM after subtracting background staining.

Table 2. Expression of Fcγ and complement receptors and other functional cell surface molecules by CD3$^-$ DGL clones. Two clones, one CD2$^+$CD56$^+$, the other CD2$^+$CD56$^-$, were not tested for FcγR or CR and have been omitted

No. of clones	CD16 (FcγRIII)	CD32 (FcγRII)	CD64 (FcγRI)	CD11b (CR3)	CD21 (CR2)	CD35 (CR1)	CD94 (Kp43)	HP3E4	CD8	CD103 (HML-1)
CD2$^+$56$^+$										
6	−	−	−	−	−	−	−	−	−	+
1	−	−	−	−	−	−	+	−	−	+
1	−	−	−	−	−	−	−	−	−	−
1	−	−	−	−	−	−	−	+	−	+
1	−	−	−	−	−	−	+	+	−	+
2	−	−	−	+	−	−	+	−	−	+
2	−	−	−	+	−	−	−	+	−	+
1	−	−	−	+	−	−	−	−	+	−
1	−	−	−	+	−	−	+	+	+	+
1	+	−	−	+	−	−	+	+	+	+
1	+	−	−	+	−	−	+	+	−	+
1	+	−	−	−	−	−	−	−	−	−
1	−	−	−	+	+	+	+	+	−	+
CD2$^-$56$^+$										
1	−	−	−	−	−	−	−	−	−	+
1	−	−	−	−	−	−	−	−	−	−
1	−	+	−	−	−	−	+	−	−	+
1	−	−	−	+	−	−	+	−	−	+
1	+	−	−	+	−	−	−	−	−	+
1	+	−	−	−	−	−	+	−	−	−
CD2$^+$56$^-$										
1	−	−	−	−	−	−	−	−	−	+
1	−	+	−	+	−	−	−	−	−	+
1	−	+	−	+	−	−	−	−	−	−
1	−	−	−	+	−	+	−	−	−	+
1	−	−	−	+	+	+	−	−	−	−
CD2$^-$56$^-$										
2	−	−	−	−	−	−	−	−	−	+

Cytoplasmic CD3ζ and CD3ε expression by cell surface CD3$^-$ DGL clones

Although none of these DGL clones displayed surface CD3 staining, cytoplasmic ε and ζ chains of the CD3 complex were readily detectable in the majority of cytospin preparations stained by immunocytochemistry (Table 3). Cytoplasmic CD3ζ chain expression was detected in all but one CD3$^-$ DGL clone, including all of those which were CD16$^+$. Five clones which were CD2$^-$CD16$^-$HP3E4$^-$ expressed CD3ζ, and three of these were also CD94$^-$ (Table 2). The single clone which was completely negative for cytoplasmic CD3ζ (Table 3) was also negative for these four markers. The level of expression varied between clones, but 68% expressed CD3ζ chain in more than 50% of all cells, and 41% of clones in more than 75% of cells (Table 3). Cytoplasmic CD3ε chain was not detectable in 12% of DGL clones, while the remainder showed variable expression of cytoplasmic CD3ε (1–75% of cells positive; Table 3). There was a clear correlation between expression of cytoplasmic CD3ε and CD3ζ chains by individual DGL clones (Table 3).

Double labelling of fresh permeabilized DGL with anti-CD56 and anti-CD3ζ or CD3ε mAb revealed expression of CD3ζ by most CD56$^+$ cells, whereas CD3ε was only detected in a small number of CD56$^+$ DGL (Fig. 3). The intensity of surface CD3 labelling was much lower in fixed and permeabilized cells (Fig. 3c) than in unfixed cells (Fig. 3a), possibly as a result of decreased antibody reactivity after fixation. Also,

Table 3. Expression of cytoplasmic CD3ε and CD3ζ chains by cell surface CD3$^-$ DGL clones*

Number of clones	Cytoplasmic CD3ε	Cytoplasmic CD3ζ
1	0	0
1	0	+
2	0	++
7	+	++
9	++	+++
13	++	++++
1	+++	++++

0, < 1% positive cells; +, 1–25% positive cells; ++, 26–50% positive cells; +++, 51–75% positive cells; ++++, > 75% positive cells.

* One clone was not tested for CD3ε or CD3ζ expression.

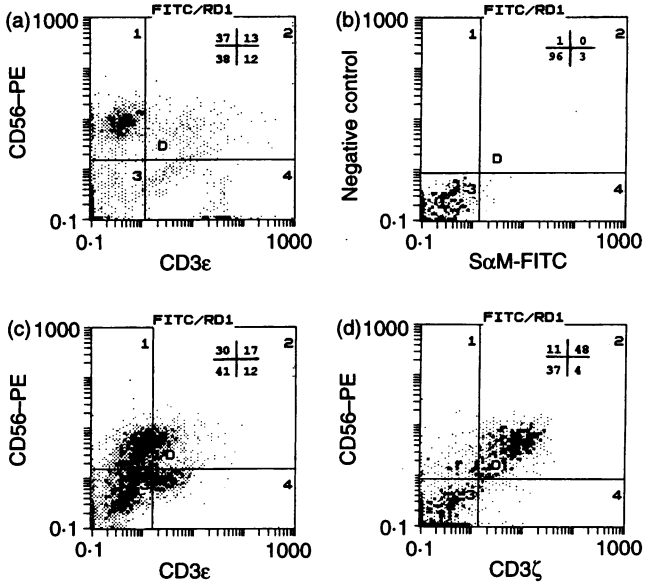

Figure 3. Representative flow cytometric profiles of cell surface and cytoplasmic antigen expression by freshly isolated decidual cells. (a) Unfixed cells; cell surface labelling for CD56 and CD3ε; (b–d) fixed and permeabilized cells; (b) negative control; cells labelled with sheep–anti-mouse immunoglobulin–fluorescein isothiocyanate alone; (c) cells surface labelled with anti-CD56-PE and for cytoplasmic CD3ε; (d) cells surface labelled with anti-CD56-PE and for cytoplasmic CD3ζ. Figures indicate that % of cells falling within quadrants 1–4. Two other decidual samples gave similar results.

cytoplasmic CD3ζ expression was almost entirely confined to $CD56^+$ cells and was apparently absent from most decidual T cells (Fig. 3d). Significant expression of cytoplasmic CD3ε and ζ chains was also found in CD3-depleted PBMC preparations cultured in IL-2 for 3 days (data not shown).

Expression of other functional molecules by $CD3^-$ DGL clones

HML-1 (CD103) was expressed by 80% of DGL clones, regardless of their expression of CD2 or CD56 antigens (Table 2), but was absent from >90% of PBNK clones (Fig. 1) and >80% of fresh decidual leucocytes (Fig. 2). Expression of CD8 molecules, however, was restricted to those clones that coexpressed CD2 and CD56. The percentage of $CD8^+$ DGL clones was similar to that of PBNK clones (Fig. 1) and to the percentage of $CD8^+$ cells in fresh decidual leucocyte populations (Fig. 2).

DISCUSSION

Comparison of the antigenic phenotype of $CD3^-$ DGL clones with that of fresh $CD3^-$ decidual leucocyte populations has revealed that they are similar (Figs. 1 and 2), indicating that the cloned cells are indeed representative of fresh $CD3^-$ DGL although proliferative frequencies of $CD3^-$ DGL were low. The main phenotypic differences between fresh $CD3^-$ DGL and DGL clones were in cell surface HML-1 (CD103) expression, as previously reported,[5] and in cytoplasmic CD3ε staining, both of which were present in a higher proportion of cloned cells than fresh DGL. A small proportion (<20%) of fresh $CD3^-$ DGL express HML-1,[20] possibly those with an intraepithelial location,[21] and it is possible that these were selectively expanded under the culture conditions employed. However, HML-1 can be induced on peripheral blood T cells activated with PHA,[22] and is maintained primarily on $CD8^+$ T cells following culture of T lymphoblasts in interleukin-2 (IL-2).[23] It is therefore more likely that expression of HML-1 was induced and maintained on some $CD3^-$ DGL clones during the culture period. It has recently been reported that E-cadherin is a ligand for the HML-1 integrin[24] and, as this molecule is expressed on endometrial epithelial cells,[25] it could function *in vivo* to retain HML-1^+ DGL within epithelial structures.

A further difference noted between $CD3^-$ DGL and PBNK clones in functional cell surface antigen expression was the significantly lower percentage of DGL clones that expressed CR3 (CD11b/CD18). It has been reported previously that fresh $CD3^-CD56^{bright}$ DGL express lower levels of CD11b than $CD3^-$ PBNK cells, and that culture of fresh DGL in IL-2 leads to down-regulation of CD11b but up-regulation of CD11a.[20] CR3 has been implicated in leucocyte adherence to endothelial cells and the extracellular matrix, and also can interact with factor X, fibronectin and CD54.[26] CR3 may enhance NK cell-mediated killing of C3bi-bearing target cells,[27] which could provide a partial explanation of the lower MHC-non-restricted killing activity by DGL than PBNK cells. Alternatively, this receptor might mediate cell–cell or cell–matrix adhesion of $CD3^-$ DGL *in vivo*. The immunobiological consequences of the absence of Fcγ receptors on the majority of $CD3^-$ DGL, and hence impaired antibody-dependent cellular cytotoxicity, in the context of materno–fetal interactions is unclear.

CD3ζ is normally associated with the CD3γδε molecular complex on T cells,[28] but is found also in association with the transmembrane form of CD16 on PBNK cells.[29] In the present study, only 14% of DGL clones were $CD16^+$ compared with 28% in our earlier report,[5] which could reflect a more efficient removal of contaminating peripheral blood leucocytes. All $CD16^+$ clones expressed the CD3ζ chain, but this molecule was also detected in the cytoplasm of all but one $CD16^-$ clone. Most of these expressed either CD2 and/or the p58 family molecule recognized by the HP3E4 mAb, with which CD3ζ might also be associated.[17,30] However, five clones were negative for CD16, CD2 and the p58 molecule recognized by HP3E4, indicating that CD3ζ may be associated with an alternative cell surface molecule in these cases, possibly one of the other members of the p58 family of molecules.[17] As most fresh $CD56^+$ DGL also contained cytoplasmic CD3ζ, its presence in $CD3^-$ DGL clones was not a result of prolonged culture in IL-2.

CD3ζ is involved in signal transduction both in T cells and NK cells[28,31] and, since it is expressed by most $CD3^-$ DGL clones, the low MHC-non-restricted cytotoxic activity of such clones cannot be explained by an absence of this molecule. Within individual $CD3^-$ DGL clones there was considerable heterogeneity in CD3ζ expression and, in some clones, only a relatively small fraction of cells contained detectable CD3ζ; those cells lacking ζ expression may have been functionally deficient in signal transducing ability. Interestingly, fresh decidual T cells apparently lacked CD3ζ expression, as has also been reported in tumour-infiltrating T lymphocytes.[32]

Cytoplasmic CD3ε was absent from most fresh $CD3^-$

decidual leucocytes, but was expressed in the majority of $CD3^-$ DGL clones. As with CD3ζ, there was heterogeneity among clones, with between 1 and 70% of cells of individual clones expressing detectable cytoplasmic CD3ε chains. Induction of cytoplasmic, but not cell surface, CD3ε expression in the absence of CD3γ and δ chains has been reported both in PBNK cells[18] and in the common thymic precursors of T cells and NK cells cultured in IL-2.[33] The epitope of CD3ε recognized by UCHT1 has been reported to be dependent upon co-ordinate expression of either CD3γ or δ,[34] suggesting that $CD3\varepsilon^+$ surface $CD3^-$ DGL clones were also expressing either CD3γ or δ in the cytoplasm. Alternatively, with the fixation and staining procedures used cytoplasmic CD3ε may have been detectable alone.

Although the majority of fresh decidual leucocytes and PBNK clones expressed CD56 and Kp43 (CD94), 60% of $CD3^-$ DGL clones were $CD94^-$. This molecule may recognize HLA-B7 on target cells, leading to transmission of a negative signal, rendering $HLA\text{-}B7^+$ target cells resistant to lysis by $CD94^+$ NK cells.[35] The relatively reduced numbers of $CD94^+$ DGL clones could have been a result of selective outgrowth or down-regulation of CD94 with culture in IL-2. An absence of CD94 might be expected to increase their repertoire of target cell lysis but, in our previous study,[5] $CD3^-$ DGL clones were poorer than PBNK clones at lysing the $HLA\text{-}B7^-$ NK cell-resistant target cell line BSM. However, CD94 is also capable of signal transduction[11] and interaction with other target cell surface molecules might facilitate triggering of lysis in a manner not possible in $CD94^-$ cells. Alternatively, $CD3^-$ DGL could lack other cell surface or cytoplasmic molecules in addition to CD16, rendering them less efficient at mediating MHC-non-restricted cytotoxicity than $CD3^-$ PBNK cells.

The p58 family molecule studied here, that recognized by the HP3E4 mAb, was also present on a lower proportion of $CD3^-$ DGL than PBL clones. Four phenotypically distinct subsets of cloned DGL were identified, namely $CD94^-HP3E4^-$ (60%), $CD94^+HP3E4^-$ (17%), $CD94^-HP3E4^+$ (9%) and $CD94^+HP3E4^+$ (14%), illustrating the extensive heterogeneity of expression of p58 family molecules by DGL. With regard to DGL function *in vivo*, it remains possible that other p58 molecules render DGL unable to kill $HLA\text{-}G^+$ fetal cytotrophoblast in the placental bed[36,37] leading to failure of potential cytolysis and contributing to the immunobiological success of human pregnancy.

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