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Characterization of decidual leukocyte populations in cynomolgus and vervet monkeys

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Abstract

The objective of this study was the phenotypic and functional evaluation of decidual immune cells in the cynomolgus and vervet monkeys. Early pregnancy (day 36-42) deciduas were obtained by fetectomy for histological evaluation and decidual mononuclear leukocyte (MNL) isolation. While peripheral NK (pNK) cells in these species do not express CD56, CD56⁺ NK cells were abundant in decidual samples. The majority of decidual NK (dNK) cells (>80%) had high light-scatter characteristics and were CD56^{bright}CD16⁺ cells with no or very low levels of natural cytotoxicity receptors (NKp46, NKp30) and NKG2A, while a minor population were small CD56^{dim}CD16⁺ lymphocytes also expressing less NKp46, NKp30 and NKG2A than pNK cells. All dNK cells were found to be perforin⁺; however, their cytotoxic potential was low and cynomolgus dNK cells showed strongly reduced cytotoxicity against target cells compared with pNK cells. Macrophages and T cells together comprised approximately 25-30% of decidual MNL. Decidual T cells contained a higher proportion of the minor T cell subtypes (γδT cells, CD56⁺ T cells) compared with peripheral blood. A subset of DC-SIGN⁺ macrophages, with a distribution adjacent to areas of placental attachment in contrast to the widespread setting of general CD68⁺ cells, was identified in both species. Together, these results demonstrate that the maternal-fetal interface in both cynomolgus and vervet monkeys is very rich in immune cells that have similar phenotypes to those seen in humans, indicating that both species are excellent models to study the contributions of distinct immune cell populations to pregnancy support.

Keywords

cynomolgus; vervet monkey; decidua; NK cell; macrophage; NK cell receptors; DC-SIGN

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Introduction

Nonhuman primates represent critical models for the study of maternal-fetal interactions of human pregnancy because of the similarity of many aspects of reproductive biology including menstrual cyclicity, placental architecture, chorionic gonadotropin gene expression, and the MHC class I profile of the trophoblasts (Golos, 2003; Carter, 2007). The rhesus monkey (*Macaca mulatta*) and the baboon (*Papio sp.*) have been models of choice in current pregnancy research in particular; however, the development of alternate primate models has been identified as a priority aim by the National Academy of Sciences (Rhesus monkey demands in biomedical research, 2003), to alleviate the dependence on access to these individual species. Two species, the cynomolgus monkey (*Macaca fascicularis*) and vervet (African green) monkey (*Chlorocebus aethiops*), are in the same subfamily as the rhesus and baboon. They have a villous placental morphology, and a hemochorial mode of placentation, as in the human and rhesus monkey (Carter, 2007).

After embryo implantation, decidualization of primate endometrium is associated with a dramatic recruitment of immunocompetent cells, including large numbers of NK cells and abundant macrophages, whereas the number of T cells is decreased (Bulmer, 1994, Slukvin et al, 2001, Trundley and Moffett, 2004). Decidual NK (dNK) cells are large granular lymphocytes, comprising up to 70% of the decidual leukocyte population, have a phenotype distinct from peripheral blood (pNK) cells (Koopman et al, 2003) and their role in the regulation of human placental development and endometrial angiogenesis is becoming more clearly delineated. Trophoblast invasion can be triggered and directed by dNK cells via IL-8 and IP-10; the secretion of vascular endothelial growth factor (VEGF) and placental growth factor (PLGF) may contribute to the pro-angiogenic properties of dNK cells (Hanna et al, 2006, Lash et al, 2006). The functional mode of human dNK cells could be influenced by their recognition of the nonclassical MHC class I molecules HLA-G and HLA-E expressed on trophoblasts in the absence of classical MHC (King et al, 2000). Macrophages were also shown to express receptors for HLA-G (McIntire et al, 2004). In 1st trimester human decidua, macrophages constitute about 20% of the total leukocytes (Starkey et al, 1988) and are reported to display a state of alternative activation with immuno-suppressive characteristics (McIntire and Hunt, 2005). An increased percentage of discrete subsets of T cells in the decidua in comparison with peripheral blood has been reported. $\gamma\delta$ T cells, which comprise about 5% of circulating T cells, were found to compose up to 25% of human decidual T cells (Mincheva-Nilsson et al, 1997). These T cells are not restricted by classical MHC class I molecules and have a high immunoregulatory potential.

We thus analyzed early pregnancy cynomolgus and vervet monkey decidua to reveal the phenotypic spectrum and histological distribution of NK cells, macrophages and T cells. Our results demonstrate that overall, cynomolgus and vervet monkeys, as models for the investigation of human maternal-fetal immune interactions, have substantial homology with the human as well as with the rhesus macaque.

Materials and methods

Animals and surgery

Female cynomolgus and vervet monkeys used for timed matings were from the colony maintained at the Wisconsin National Primate Research Center. Decidual tissues were obtained by fetectomy at selected stages of pregnancy. All surgical procedures were performed in accordance with the NIH Guide for the Care and Use of Laboratory Animals and under the approval of the University of the Wisconsin Graduate School Animal Care and Use Committee.

Immunohistochemistry (IHC)

Decidual tissues were embedded in OCT mounting medium (Sakura Finetek) and frozen in isopentane cooled with dry ice and ethanol. Seven μm sections were cut, treated with hydrogen peroxide/methanol, blocked with the appropriate 20% serum matched to the secondary antibody, rinsed with PBS, and incubated with anti-CD3 (clone FN-18, Biosource), CD56 (MY31, Becton Dickinson), CD68 (EBM11, Dako), DC-SIGN (DCN46, BD Pharmingen) or the corresponding isotype controls. Vectastain ABC peroxidase complex and NovaRed substrate (Vector Laboratories) were used for positive immunostaining visualization.

Decidual and peripheral MNL isolation

Decidual tissue was dissected free of the amniotic membranes and placenta, and minced. Digestion was performed with a collagenase IV/DNAse cocktail as previously described (Slukvin et al., 2001). MNL from the suspension of decidual cells and from heparinized venous blood were separated on a gradient of Ficoll-Paque Plus solution (GE Healthcare).

Flow cytometry

mAbs used in analysis are listed in Table 1. Cells were incubated with the corresponding mAbs for 30 min at 4°C in PBS with 2% fetal bovine serum (FBS) (Atlanta Biologicals), washed and fixed with 2% paraformaldehyde. Control cells were stained with isotype, concentration and fluorochrome matched mouse IgG. At least 10,000 events for each sample were collected and analyzed, using FACSCalibur and FlowJo software (Becton Dickinson, Tree Star). Intracellular perforin staining was performed on cells prestained with anti-CD56-PE-Cy5 and fixed/permeabilized with Cytotfix/Cytoperm solution (BD Biosciences).

A DC-SIGN-enriched cell suspension was obtained from decidual MNL after anti-DC-SIGN-FITC labeling followed by positive selection using anti-FITC microbeads (Miltenyi Biotec).

Cytotoxicity assay

The cytolytic activity of dNK and pNK cells was tested in a ^{51}Cr -release assay as previously described (Slukvin et al., 2001). Anti-CD56 microbeads (Miltenyi Biotec) were used to separate dNK cells from decidual MNL. Enriched pNK cells were isolated via negative selection by labeling peripheral blood MNL with PE-conjugated mAbs to CD3, CD20, CD14, followed by anti-PE microbeads (Miltenyi Biotec) and magnetic depletion.

Results

Morphological analysis of immune cell localization in decidua

Resident leukocytes within the decidual tissue were defined by IHC (Fig. 1) and flow cytometry (below). Fig. 1 illustrates that in both cynomolgus and vervet decidua numerous cells expressed CD56 (likely NK cells), CD68 (macrophages), and DC-SIGN (macrophages or dendritic cells). The widespread distribution of CD56⁺ cells, and the relative scarcity of CD3⁺ T cells (Fig. 1A) is highly reminiscent of rhesus monkey decidua in early pregnancy (Slukvin et al., 2001). In addition, the morphological appearance of CD68⁺ and DC-SIGN⁺ cells in cynomolgus and vervet (Fig. 1B) is very similar to that seen in rhesus decidua (Brebuda et al., 2006). CD68 cells were evenly distributed throughout the decidua, with a more rounded appearance, likely because of its intracellular localization (Saito et al., 1991). Contrary to CD68, DC-SIGN IHC revealed cells with dendritic-like processes and a distribution pattern restricted to areas of villous and extravillous trophoblast attachment (Fig. 1B).

Phenotype of pNK cells

We next examined the phenotype of pNK cells in cynomolgus and rhesus monkeys. As anticipated based on our previous studies (Slukvin et al., 2001) and others in the literature (Yoshino N. et al, 2000, Holznagel et al, 2002), in both species pNK cells gated as CD3⁺CD16⁺ lymphocytes were CD56⁻ (Fig. 2A). In cynomolgus monkeys, like rhesus monkeys, CD56 was expressed by peripheral blood CD14⁺ monocytes, whereas in rhesus monkeys the monocytes were CD56⁻ (Fig. 2B).

Phenotype of dNK cells

We evaluated the phenotype of decidual leukocytes. A large number were CD56^{bright} cells with high light-scatter characteristics implying large granular cells (e.g., Fig. 3A (gate R1)), similar to the CD56^{bright} population of dNK cells in the human and rhesus monkey decidua (Koopman et al., 2003, Slukvin et al., 2001). In addition a minor population with a low light-scatter profile and CD56^{dim}CD3⁻ phenotype, was identified in both cynomolgus and rhesus monkey decidual cell suspensions (Fig. 3A (gate R2)), as noted previously in rhesus (Slukvin et al, 2001). These cells comprised 13.2±8.5% of the total CD56⁺ decidual cells in cynomolgus (n=9) and 13.4±8.4% in rhesus monkeys (n=6). Cynomolgus and rhesus monkey decidual CD56^{bright} and CD56^{dim} cells were CD45⁺, and had a typical phenotype of nonhuman primate NK cells: CD3⁺CD14⁺CD16⁺CD8⁺ (both positive and negative staining was detected for CD16 and CD8) (Table 2, Fig. 3A) and were designated as CD56^{bright} and CD56^{dim} dNK cells. While both populations of dNK cells were distinct from pNK cells in their diminished expression of CD8 and CD16, within the CD56^{dim} dNK population the percent of CD16⁺ cells was notably low (Table 2). The activation marker, CD69, was detected on a proportion of dNK cells in both species (Table 2). CD69 may have been acquired in vitro during the cell isolation procedure, as shown previously in human for CD56^{bright} cells (Vassiliadou and Bulmer, 1998). However, the percentage of cells co-expressing CD69 was consistently higher in CD56^{dim} than in CD56^{bright} cells, indicating possible higher responsiveness of the CD56^{dim} population to purification steps, or perhaps a true in vivo phenotype. In cynomolgus monkeys, lower expression of the CD45RA antigen was detected on dNK cells compared to pNK cells that were uniformly CD45RA⁺ (Table 2).

NK cell subsets from cynomolgus monkeys were analyzed in addition for reactivity with anti-human NKp46, NKp30 and NKG2A mAbs (Fig. 3C). Expression of NCRs, which are activating receptors, and NKG2A, which associates with CD94 to form an inhibitory NK cell receptor, were detected on pNK cells and in lower level on CD56^{dim} dNK cells. CD56^{bright} dNK had no or very low expression of NKp30, NKp46 and NKG2A. We evaluated the cytotoxicity of cynomolgus NK cells against K562 and 721.221 MHC class I null target cells, and revealed low cytotoxicity of dNK cells in comparison with pNK cells (Fig. 4B), despite perforin expression in the former (Fig. 4A).

Phenotype of decidual macrophages

IHC (Fig. 1B) and flow cytometry analysis (Fig. 3A,B) revealed that macrophages constituted the second major leukocyte population in 1st trimester rhesus and cynomolgus decidua. In five cynomolgus monkeys CD14⁺ macrophages made up 14.6±4.2% of decidual MNL (Fig. 3B). Macrophages in two rhesus monkeys [19.1% and 38.7% of total decidual MNL (not shown)] were unexpectedly in higher numbers than CD56^{bright} dNK cells (17.8±8.8%, average data of five samples) (Fig. 3B). Decidual macrophages in both species can be detected via CD64 (FcγRI) and HLA-DR, and to some extent by CD206 (mannose receptor) (Fig. 5A,B). DC-SIGN⁺ cells were found to co-express CD14, CD64 and HLA-DR (Fig. 5A, B). Detailed analysis of the DC-SIGN-enriched cell preparation verified that all DC-SIGN⁺ cells express the macrophage-specific marker CD14 (Fig. 5C), whereas no dendritic cell-specific markers were detected (CD86, CD83, not shown).

Phenotype of decidual T cells

We also identified T cells in cynomolgus and vervet monkey decidual tissues (Fig. 3A, B). Cytotoxic (CD8⁺) T cells were prevalent in the cynomolgus monkey tissues, while the percentage of CD4⁺ T cells was lower when compared with peripheral blood (Table 3). In the decidua only small numbers of CD45RA⁺ T cells, which are considered to be naïve T cells, were present (Table 3), implying possible predominance of antigen-committed memory T cells. The expression by decidual T cells of CD69 (Table 3) was similar to human studies, where T cells constitutively express CD69, which was not acquired during the purification procedure (Vassiliadou and Bulmer, 1998).

T cells co-expressing the NK cell marker CD56 constitute on the average 7.9% (n=9) of decidual T cells in cynomolgus and 4% (n=4) in vervet monkey decidua, while within peripheral T cells the percent of CD3⁺CD56⁺ cells is <1% (Table 3). The higher proportion of $\gamma\delta$ TCR expression among CD3⁺ cells in decidua than in peripheral blood was seen in cynomolgus monkey (Table 3). Analogous to the human decidua (Mincheva-Nilsson et al, 1997), cynomolgus and vervet monkey decidual $\gamma\delta$ T cells comprised a heterogeneous population of CD56⁺ and CD56⁻ cells (Fig. 6).

Discussion

In this study we demonstrated that as with human and the rhesus monkey, NK cells and macrophages are abundant in the cynomolgus and vervet monkey decidua. Phenotypic analysis of dNK cells in these species revealed clear differences from pNK cells, especially in CD56 expression. dNK cells also have a lower level of expression of CD8, CD16 and CD45RA. The level of CD45RA and CD45RO on human pNK cells was reported to display the NK cell activation state (Lima et al., 2002a). Downregulation of CD45RA and upregulation of CD45RO were associated with acute viral infections, while in healthy donor blood the majority of pNK cells are CD45RA⁺CD45RO⁻ (Lima et al., 2002a,b). Cynomolgus monkey pNK cells had almost uniformly high CD45RA staining. In contrast, dNK cells in cynomolgus and vervet monkeys showed very low of CD45RA expression, pointing to possible activation of dNK cells. Unfortunately, the evaluation of CD45RO expression in these monkeys was not feasible due the lack of specific mAbs. Interestingly, the NK cell population isolated from early pregnancy cynomolgus and vervet decidua consists of two unequal subsets: the majority of dNK cells are large granular CD56^{bright} cells which are primarily CD16⁺, and the minor subset is represented by small cells with a CD56^{dim}CD16⁻ phenotype. In both species CD56^{dim} cells showed consistently higher CD69 expression than CD56^{bright} cells, indicating possible higher responsiveness of the CD56^{dim} population to purification steps (Vassiliadou and Bulmer, 1998) or perhaps true in vivo expression.

Additional analysis of cynomolgus NK cells revealed further differences between dNK and pNK cells. High expression of NKp46, NKp30 and NKG2A is a characteristic of pNK cells, while low expression was detected on dNK cells. Interestingly, dNK cell subsets had different patterns of expression of these receptors. All three receptors were detected only in the minor CD56^{dim} subset. CD56^{bright} cells showed low expression of NKp46, but no or very low expression of NKp30 and NKG2A. The observed heterogeneity of dNK cells likely mirrors their developmental stage and/or origin. The subsets might represent distinct developmental stages within NK cells originating in the endometrium, distinct stages of local differentiation of pNK cells following migration into the endometrium, or the subsets could represent a combination of these two alternative populations. The origin of dNK cells in the human endometrium remains incompletely understood. It was reported that NK cells migrate to the decidua from peripheral blood via their chemokine receptors (Hanna et al., 2003, Carlino et al., 2008). Alternatively, they may differentiate in situ from endometrial

CD34⁺ NK cell precursors (Keskin et al., 2007). Recently it was discovered that NK cells in human non-pregnant endometrium are relatively inactive, have a unique phenotype (in that they are NKp30⁻), and were proposed as immature cells awaiting pregnancy (Manaster et al., 2008). The differential expression of NCRs between CD56^{bright} and CD56^{dim} dNK subsets with the CD56^{bright} dNK cell subset being exclusively NKp30⁻ was also observed in rhesus monkey (unpublished data). The lack of NKp30 on CD56^{bright} dNK cells could be consistent with a local origin of this unique NK cell population. Whereas, CD56^{dim} dNK cells, which are NKp30⁺, may traffic from peripheral blood but may represent the next developmental step after the CD56^{bright} stage.

It is worth noting that divergent responses to engagement of NKp46 and NKp30 in the total human dNK cell population have been reported (El Costa et al., 2008). NKp46 mediates dNK cytotoxicity, while triggering of NKp30 induces proinflammatory cytokine secretion, although both responses were well controlled by NKG2A inhibitory receptors. Our discovery of differential expression of NKp46 and NKp30 on macaque dNK cells could help define a role these receptors may have in this unique tissue environment. At the present time the expression of an undefined NKp30 ligand has already been described in human decidua (Hanna et al., 2006).

Decidual macrophages are second in abundance to dNK cells in cynomolgus and possibly more abundant than dNK cells in vervet monkey decidual tissues, and include a DC-SIGN⁺ population that is distinct from dendritic cells. DC-SIGN is highly expressed on dendritic cells in mucosal tissues and is essential to the specific migratory function of these cells (Steinman, 2000). It was reported that DC-SIGN is highly expressed on macrophages in human lymph nodes (Granelli-Piperno et al., 2005). Our previous analysis of rhesus decidual MNL showed that DC-SIGN⁺ cells co-express several macrophage markers including CD14 and CD64 (Brebura et al., 2006). This macrophage population was detectable only in pregnant and not nonpregnant endometrium and the appearance of DC-SIGN⁺ cells in vervet and cynomolgus monkey decidual tissue is an intriguing parallel to the rhesus that supports our hypothesis that DC-SIGN may be a selective marker of the immunological response of pregnancy (Brebura et al., 2006). The role of DC-SIGN at the maternal-fetal interface is not defined, but could include conferring an enhanced ability of pathogen recognition, or a higher migratory ability of DC-SIGN⁺ cells.

Flow analysis of decidual macrophages in these species also revealed a high proportion of CD206⁺ macrophages. CD206 is an endocytic receptor as well as a mediator of phagocytosis, it is important in removing microorganisms and apoptotic cells, and in tissue remodeling (Taylor et al., 2005). In human first trimester decidua, CD206 was detected on macrophages surrounding glands, in contrast to disseminated distribution in term placenta (Laskarin et al., 2005). Expression of CD206 is a characteristic of the alternative activation of macrophages, when they promote cell growth and tissue repair, in contrast to classical activation accompanied by a proinflammatory response (Taylor et al., 2005).

Finally, although decidual CD3⁺ T cells are comparatively low in abundance, they also have distinct phenotypic populations. Unlike in blood, cytotoxic CD8⁺ T cells are found to be prevalent in the cynomolgus decidua. The predominance of CD8⁺ T cells also was reported in human early pregnancy decidua where they were shown to produce IFN- γ and IL-8 and may regulate extravillous invasion (Scaife et al, 2006). Minor T cell subsets ($\gamma\delta$ T cells and CD3⁺CD56⁺ cells) were in higher proportions in monkey decidua than in peripheral blood. It is proposed that decidual $\gamma\delta$ T cells play an important role in the maintenance of normal human pregnancy through the expression of immunoregulatory cytokines TGF- β and IL-10 (Nagaeva et al., 2002). In the mouse it was shown that $\gamma\delta$ T cells are critical for the Th2 bias (Arck et al, 1997). Most of the defined ligands of the $\gamma\delta$ TCR are molecules indicating

cellular stress (CD1, MICA, MICB) (Born et al, 2006). CD1 expression was demonstrated in the human implantation site on both villous and extravillous trophoblasts (Boyson et al., 2002) and perhaps at the fetal-maternal interface other ligands remain to be identified. Another minor T cell subset, not analyzed in the current study, iNKT cells, also was reported to be prevalent in human decidua in comparison to peripheral blood (Boyson et al., 2002, Tsuda et al., 2001). A significant immunoregulatory role of iNKT cells was indicated by production of IFN- γ . Our observation of increased CD3⁺CD56⁺ cells in the decidua versus peripheral blood requires additional analysis to determine a phenotype and the role they play at the maternal-fetal interface.

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Fig. 1.

Immunohistochemical (IHC) analysis of cynomolgus decidua at d42 and vervet decidua at d37 of pregnancy. **(A)** Representative staining is shown for the CD56, CD3 antibodies. NS indicates nonspecific primary antibody. **(B)** Representative staining is shown for the CD68 and DC-SIGN antibodies. “V” indicates placental villi. Scale bar: 100 μ m.

Fig. 2. Phenotype of cynomolgus and vervet monkey peripheral NK cells (**A**) and monocytes (**B**). Lymphocytes and monocytes were gated according to light-scattering characteristics (not shown). The expression of CD16 and CD56 on lymphocytes and the expression of CD14 and CD56 on monocytes are shown.

Fig. 3.

Immune cells in 1st trimester cynomolgus and vervet monkey endometrium. **(A)** Decidual leukocyte cells were stained with directly labeled mAbs for CD56, CD14, and CD3. Decidual leukocytes are composed of two distinct fractions according to their light-scattering characteristics (FSC/SSC dot plot) – region gate R1 and region gate R2. Macrophages and CD56^{bright} cells were detected within R1, while T cells and CD56^{dim} cells were detected within R2. Results of representative analysis of cynomolgus decidual leukocyte suspension are shown. **(B)** Numbers of dNK cells (CD56^{bright} and CD56^{dim}), T cells (CD3⁺) and macrophages (CD14⁺) were determined and related to the number of MNL isolated from monkey decidua. Data represent the mean $\pm$ S.D. values of 3-9 separate

experiments. * = macrophage data are not included, see text. (C) Staining of cynomolgus peripheral and decidual NK cells with NCR and NKG2A antibodies. pNK cells were gated on the CD3⁺CD16⁺ cells within the lymphocyte gate, CD56^{bright} dNK cells were gated on the CD56⁺CD14⁻ cells within R1 gate, and CD56^{dim} dNK cells were gated on the CD56⁺CD3⁻ cells within R2 gate. Filled histograms represent staining with the indicated mAbs, open histograms are isotype control staining.

Fig. 4. Cytotoxic activity of peripheral and decidual NK cells in cynomolgus and vervet monkey. **(A)** Decidual leukocyte cells were stained with mAbs for CD56 and perforin or isotype control mAb. Analysis of the CD56⁺ cells is shown. Filled histograms represent staining with perforin, open histograms are isotype control staining. **(B)** ⁵¹Cr-release assay for cynomolgus CD56⁺ cells isolated from 1st trimester decidua and pNK cells from the same animal against classical MHC class I-negative target cells (K562 and 721.221). Effector:target ratio is indicated on the x-axis axis. Asterisk (*) indicates statistical significance as determined by Student's t test ($P < 0.05$). Data represent the mean $\pm$ S.D. values of three separate experiments.

Fig. 5.

Flow cytometry analysis of cynomolgus and vervet monkey decidual macrophages. Decidual leukocyte cells were stained with mAbs for CD14, CD64, DC-SIGN (or CD206) and HLA-DR. Dot plots represent the fluorescence for corresponding mAbs of R1 gated cells (Fig.3A). (A) Cynomolgus monkey decidual leukocyte analysis (top row). (B). Vervet monkey decidual leukocyte analysis (second row). (C) The analysis of the DC-SIGN⁺ cell-enriched population isolated from vervet monkey decidua.

Fig. 6. CD56 and $\gamma\delta$ TCR expression in decidual CD3⁺ T cells gated within the R2 gate (Fig. 3A) in cynomolgus and vervet monkeys. Decidual leukocytes were stained with mAbs for CD3, CD56, and $\gamma\delta$ TCR. Control samples were stained with isotype matched nonspecific mAbs.

Table 1

MAbs used in flow cytometry analysis of immune cell populations in cynomolgus and vervet early pregnancy decidua.

Antigen-format	Clone	Isotype	Supplier
CD3-PE	SP34	Mouse IgG3	BD Pharmingen
CD3-PE-Cy7	SP34-2	Mouse IgG1	BD Pharmingen
CD4-PE	M-T477	Mouse IgG2a	BD Pharmingen
CD8-PE	RPA-T8	Mouse IgG1	BD Pharmingen
CD8-APC	DK25	Mouse IgG1	DakoCytomation
CD14-FITC, APC	M5E2	Mouse IgG2a	BD Pharmingen
CD16-FITC	3G8	Mouse IgG1	BD Pharmingen
CD16-PE	DJ130c	Mouse IgG1	DakoCytomation
CD45-APC	MB46D6	Mouse IgG1	Miltenyi Biotec
CD45RA-PE	5H9	Mouse IgG1	BD Pharmingen
CD56-PE-Cy5	B159	Mouse IgG1	BD Pharmingen
CD64-PE	10.1	Mouse IgG1	BD Pharmingen
CD69 PE	FN50	Mouse IgG1	BD Pharmingen
CD83-FITC	HB15a	Mouse IgG2b	Immunothech
CD86-FITC	FUN1	Mouse IgG1	BD Pharmingen
CD206-FITC	19.2	Mouse IgG1	BD Pharmingen
DC-SIGN-FITC	DCN46	Mouse IgG2b	BD Pharmingen
HLA-DR-PE, PerCP-Cy5.5	G46-6	Mouse IgG2a	BD Pharmingen
NKG2A-PE	Z199	Mouse IgG2b	Immunotech
NKp30-PE	AF29-4D12	Mouse IgG1	Miltenyi Biotec
NKp46-PE	BAB281	Mouse IgG1	Beckman Coulter
TCR $\gamma\delta$ -PE	B1	Mouse IgG1	BD Pharmingen
Perforin-FITC	Pf-344	Mouse IgG1	Mabtech

Table 2

Immunophenotype of cynomolgus and vervet monkey dNK cells (CD56^{bright} and CD56^{dim} populations) and peripheral CD16⁺ cells.

	Cynomolgus				Vervet		
	decidual CD56 ^{bright}	decidual CD56 ^{dim}	peripheral CD16 ⁺		decidual CD56 ^{bright}	decidual CD56 ^{dim}	peripheral CD16 ⁺
CD8	36.9±35.9	57.8±27.9	84.2±13.4		55.5±36.7	55.9±9.1	70.4 ±41.9
CD16	85.0±10.0	20.8±14.5	100		83.1±7.3	18.6±6.7	100
CD45	100	100	100		100	100	100
CD45RA	1.4±1.5	10.3±11.7	97.0±2.6		42.7±16.8	3.3±2.7	nd
CD69	1.8±1.8	47.1±29.5	0.4±0.3		38.2±11.7	62.7±0.2	nd

The results are expressed as a mean of positive cells (%) ± SD of three to nine animals. nd = not done.

Table 3

Immunophenotype of cynomolgus and vervet monkey decidual and peripheral T cells.

	Cynomolgus		Vervet	
	decidual CD3 ⁺	peripheral CD3 ⁺	decidual CD3 ⁺	peripheral CD3 ⁺
CD4	23.8±4.0	53.5±11.2	3.5±1.6	nd
CD8	71.0±12.7	49.6±11.9	48.1±24.7	65.3±13.2
CD45RA	8.0±13.4	61.2±23.2	3.4±2.3	nd
CD56	7.9±4.7	0.4±0.2	4.0±2.9	0.4±0.3
CD69	20.5±27.7	0.5±0.5	15.3±6.4	nd
TCRγδ	5.5±2.1	1.9±1.0	3.7±1.6	nd

The results are expressed as a mean of positive cells (%) ± SD of three to nine animals. nd = not done.