Phenotypic Markers for the Feline Monocyte: Rosette Formation with Human Erythrocytes and a Monoclonal Antibody Which Binds Myeloid Cells 43262

C. E. WHITEHURST,* R. J. HILL,† N. K. DAY,† AND N. GENGOZIAN†,[1]

Department of Pediatrics,† All Children's Hospital, University of South Florida, St. Petersburg, Florida 33701 and Department of Biology, University of South Florida, Tampa, Florida 33612*

Abstract. **A small population of cells with the ability to form rosettes with human erythrocytes was found in feline peripheral blood leukocytes (PBL) (10%) and bone marrow (9%), but not in purified granulocyte preparations, thymus, and lymph node tissues. The morphologic appearance and ability to phagocytize latex beads indicated these cells were monocytes. A monoclonal antibody, CM277, with a binding specificity for feline peripheral blood phagocytes was also characterized. Immunofluorescent microscopy revealed CM277 to bind specifically to monocytes and polymorphonuclear neutrophils. The binding of CM277 to monocytes was also shown by human erythrocyte-rosette formation wherein there was a high degree of correlation between these two phenotypic markers for cells ingesting latex beads. Monocytes, polymorphonuclear neutrophils, and T lymphocytes of the cat rosette with guinea pig erythrocytes (GPE) and using CM277 we were able to determine the contribution of the former two cell types to the GPE-rosetting population. Monocytes and polymorphonuclear neutrophils comprised the majority of the GPE-rosetting cells in fresh PBL (>60%), but after culturing overnight, there was a substantial decrease in these cells (<35%). In contrast, GPE-rosetting T lymphocytes comprised approximately 10% of the cells in fresh PBL, and after *in vitro* culture for 1 day they constituted 35–45% of all cells. The removal of monocytes by human erythrocyte-rosetting did not affect the pokeweed mitogen-induced synthesis of Ig, but did lead to an increased production of interleukin 2. Removal of the GPE-rosetting population from PBL resulted in a marked decrease in interleukin 2 production, pointing to a positive contribution of GPE-rosetting T lymphocytes to the synthesis of this lymphokine.** [P.S.E.B.M. 1991, Vol 197]

The paucity of markers allowing the identification, enumeration, and fractionation of feline immune cells has made investigations of feline immunobiology difficult. To facilitate studies of the feline immune system, our laboratory has been interested in characterizing new markers for identifying and manipulating different cell populations. Guinea pig erythrocyte (GPE)-rosetting cells found in peripheral blood leukocytes (PBL) of the cat were for many years viewed as T lymphocytes (1–5). Studies in our laboratory, however, have shown that the GPE was in reality identifying the T helper subset; furthermore, additional erythrocyte-rosetting analyses revealed that the gerbil erythrocyte (GE) bound specifically to cells of the T suppressor subset (6). A question concerning the T cell specificity of the GPE-rosetting population has been raised by Wellman *et al.* (7) who reported that GPE also attached to monocytes and polymorphonuclear neutrophils (PMN), a finding confirmed in our laboratory. To clarify the contributory role, therefore, of monocytes, PMN and T lymphocytes to the GPE-rosetting population, we searched for means of identifying and isolating feline monocytes from PBL. In this report we describe two markers which meet these criteria: the human erythrocyte (HE), which rosettes specifically with cat monocytes, and a monoclonal antibody (MoAb), CM277, which binds to both monocytes

[1] To whom requests for reprints should be addressed at All Children's Hospital, Department of Pediatrics, 801 Sixth St. South, St. Petersburg, FL 33701.

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and PMN. In addition to providing a more precise definition of cells contained within the GPE-rosetting population, the present findings have also allowed us to examine the role of monocytes in two *in vitro* immune function assays of feline PBL, i.e., the pokeweed mitogen-induced secretion of Ig and the production of interleukin 2 following concanavalin A stimulation. Preliminary results have been reported (8).

Materials and Methods

Isolation of Peripheral Blood Leukocytes and Polymorphonuclear Neutrophils. Blood samples were collected from healthy adult cats by jugular venipuncture. The blood was defibrinated with glass beads and then centrifuged on a Ficoll-Hypaque gradient. The mononuclear cell band was washed with incomplete Hanks' balanced salt solution (iHBSS) and suspended in iHBSS containing 0.5% bovine serum albumin (iHBSS-BSA). Polymorphonuclear neutrophils (PMN) were also collected from Ficoll-Hypaque gradients. After centrifugation of the whole blood sample and removal of the mononuclear cells as described, the remaining interface was partially aspirated and the buffy coat on the erythrocyte pellet collected. These cells were resuspended in iHBSS and centrifuged on a Ficoll-Hypaque gradient. The top band of residual mononuclear cells was aspirated and the center PMN layer above the pelleted erythrocytes was removed and washed with iHBSS. Contaminating erythrocytes were hypotonically lysed using a K_2CO_3-buffered NH_4Cl solution. The percentage of PMN was always 90% or more as determined by Wright's staining. The viability of PBL and PMN by trypan blue exclusion was over 95%.

PBL used on the day they were obtained from the animal are referred to as fresh or Day 0 cells. Aliquots of PBL were also cultured overnight at 37°C in a 10% CO_2 humidified incubator in RPMI 1640 containing 2 m*M* L-glutamine, 10% heat-inactivated FCS, 100 units of penicillin/ml, and 100 μg of streptomycin/ml (RPMI 1640 FCS). The following day the cells were washed and resuspended in fresh medium; these cells are referred to as Day 1 cells.

Use of Frozen Tissues. Bone marrow, thymus, and lymph node tissues from kittens less than 15 weeks of age had been previously frozen in liquid nitrogen with 20% dimethyl sulfoxide. These tissues were thawed in a 37°C water bath, treated with M199 containing 40 units of DNAase/ml (Sigma Chemical Co., St. Louis, MO), and then washed in M199. Dead cells were removed by centrifugation on a Ficoll-Hypaque gradient.

Erythrocyte-Rosetting Assays. Erythrocytes (E) from humans, guinea pigs, and gerbils were collected into sterile heparinized saline and washed thoroughly with 0.15 *M* NaCl before use. To enhance GPE-rosetting, GPE were treated with *Vibrio cholerae* neuraminidase (Gibco) following the method of Gengozian *et al.* (6). Neuraminidase treatment of HE and GE was not required to obtain maximal rosetting with these cells. The percentage of HE-, GPE- and GE-rosetting cells in PBl and PMN samples was determined as follows: 100 μl of cells (3×10^6/ml) in iHBSS-BSA were mixed with 100 μl of a 1% solution of erythrocytes in iHBSS-BSA, incubated for 10 min in a 37°C water bath, and centrifuged at 200*g* for 5 min. After incubating on ice for 1 hr, the percentage of rosetting cells was determined by light microscopy using 0.04% gentian violet. At least 200 cells were counted in each assay, and a cell was scored as rosetting if three or more erythrocytes were bound to its surface. When maximal expression of GPE-rosetting capabilities of PBL was desired, the cells were cultured overnight in RPMI 1640-FCS in a 10% CO_2 humidified incubator at 37°C. After culturing, the cells were washed and resuspended in the desired medium.

Labeling of Phagocytes with Latex Beads. PBL were adjusted to 3×10^6/ml in complete Hanks' balanced salt solution containing 10% FCS. To these cells 20 μl of 0.81-μm latex beads (Bacto; Difco Laboratories, Detroit, MI) were added, and the suspension was rotated in a 37°C incubator for 90 min. The cells were washed in phosphate-buffered saline containing 20 m*M* EDTA and 0.02% NaN_3 (pH 7.2), and resuspended in iHBSS-BSA for microscopic analyses. A phagocyte was scored when three or more beads were localized within a cell.

Immunofluorescent Staining of Cells. Cells were added to microtiter plates (0.5×10^6/well), washed once with phosphate-buffered saline containing 0.01% NaN_3, and the antibody reagents added. B lymphocytes were stained using 50 μl of a 1/20 dilution of fluorescein isothiocyanate (FITC)-conjugated rabbit anti-cat IgG antiserum (Cooper Biomedical, Malvern, PA). For indirected staining with monoclonal antibodies, 100 μl of the respective hybridoma supernatants were added to the cells and the suspension was placed at 4°C for 45 min. After washing, 50 μl of a 1/10 dilution of FITC goat $F(ab')_2$ anti-mouse IgG + IgM (Tago Inc., Burlingame, CA) were added and the cells incubated as before. The cells were washed and resuspended in phosphate-buffered saline containing 0.01% NaN_3 and 0.5% BSA. Cytofluorometry was performed using a Coulter EPICS-C flow cytometer. Differing cell types were "gated" for quantification by adjusting the 90° light scatter versus forward light scatter histogram parameters. Control cells were treated with FITC-labeled goat anti-mouse antibodies. A minimum of 3000 cells were counted for each parameter. Fluorescent microscopy was performed using a Zeiss Universal microscope equipped with a xenon lamp and FITC filter. For assays involving fluorescent microscopy, at least 200 cells were counted.

Leukocyte/E-Rosette Depletions. To perform E-rosette depletions of leukocytes, PBL (3×10^6/ml) were mixed with an equal volume of 2% HE, GPE, or GE. The cell mixtures were incubated in a 37°C water bath for 15 min and then pelleted at 200*g* for 10 min. After an incubation on ice for 1 hr, the pellet was gently resuspended and the cell mixture centrifuged on a Ficoll-Hypaque gradient at 500*g* for 10 min. The non-rosetting PBL band was collected and washed with iHBSS and resuspended in iHBSS-BSA or RPMI 1640 containing 10% FCS for subsequent assays.

Reverse Hemolytic Plaque Assay for Ig Synthesis. The measurement of pokeweed mitogen (PWM)-induced polyclonal Ig synthesis was performed using the reverse hemolytic plaque assay (RHPA) (6, 9). In brief, 0.5 μg of PWM in a 50-μl volume was added to 1 ml of PBL at 1×10^6/ml. After cultivation for 6 days in a humidified 10% CO_2 incubator at 37°C, the cells were washed with HBSS and analysis for plaque-forming cells (PFC) was performed using protein-A coated sheep RBC.

Induction and Assay for Interleukin 2 Production. The optimal conditions for maximum production of interleukin 2 (IL-2) by cat PBL were found to be stimulation with 20 μg of concanavalin A per 1×10^6 cells in 1 ml of medium (RPMI 1640-FCS) cultured in flat-bottomed tissue culture wells for 24 hr (10). The assay for IL-2 was made using the IL-2-dependent murine HT-2 cell line (11). In brief, 2-fold serial dilutions of 0.1 ml of each supernatant sample were made and placed in 96-well microplates (Costar 3596; Costar, Cambridge, MA). To the diluted test samples, 0.1 ml of the assay target cells (5×10^4/ml) was added and the plates were incubated at 37°C in a humidified atmosphere of 5% CO_2 for 18 hr. Tritiated thymidine (1.0 μCi) was then added to each microculture and the cells cultured for an additional 4 hr. The cultures were harvested onto glass filter strips and the [^{3}H]thymidine incorporation (cpm) was determined in a liquid scintillation counter. The maximum radiolabel incorporation generated by a 100% control IL-2 standard (Collaborative Research, Bedford, MA) was used to calculate each datum point in terms of this control and the values plotted on probit paper to provide a linear slope with samples of increasing dilution. The X-axis dilution coordinate at which this slope crossed the 50% maximum proliferative activity, the Y-coordinate, was then defined as the unit of activity. All cultures for IL-2 production were made in duplicate, with maximum variation of 10–15% between samples. Statistical analysis of the data from four replicate experiments was made with Student's *t* test for small samples.

Results

Feline Monocytes Form Rosettes with Human Erythrocytes. A small fraction of cat PBL was found to form E-rosettes with HE. In blood samples from 12 animals, HE-rosetting cells formed an average of 10.0 ± 6.4% of all PBL. HE-rosetting cells comprised approximately 8.6 ± 3.4% of cells in the bone marrow and were found in less than 1% of all cells scored in the thymus, lymph node tissues, and purified (>90%) PMN fractions (data not shown). Wright's staining of cytospin preparations of PBL showed that the HE-rosetting cells were not lymphocytes, but rather, had a morphology like that of monocytes. They were relatively large in size with a lobulated nucleus and vacuolated cytoplasm. Since phagocytosis of latex beads is an effective marker for peripheral blood monocytes (12, 13), we examined the correlation between HE-rosette formation and the ingestion of beads. PBL were incubated with latex beads to label the monocytes and afterward mixed with HE. Using both dark field and light microscopy we found that virtually all of the HE-rosetting cells contained phagocytized beads. Photomicrographs of HE-rosetting cells containing the internalized latex beads are shown in Figure 1.

Monoclonal Antibody CM277 Binds to Feline Monocytes and PMN. A series of antibody-producing hybridoma cell lines were previously developed in our laboratory (6). One cell line, designated CM277, was found to produce an antibody with a binding specificity for feline peripheral blood phagocytes. This was determined by correlating CM277 staining of cells with the phagocytosis of latex beads. As shown in Table I, using immunofluorescent microscopy, it was found that 96.4

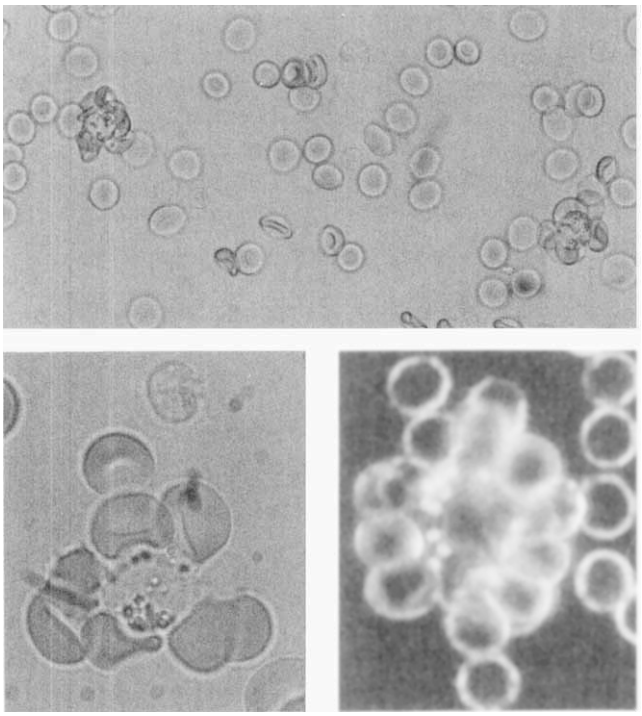

Figure 1. Photomicrographs of HE-rosetting cells which contain phagocytized latex beads.

Table I. Correlation of MoAb CM277 Staining with Bead-Ingesting Phagocytes

Experiment	PMN[a] (%)	HE rosettes (%)	Bead[b] ingesting (%)	CM277 + (%)	Beads + CM277[c] / Total beads (%)	Beads + CM277[d] / Total CM277 (%)
1	7	7	9	13	93	93
2	2	9	15	12	97	96
3	8	12	14	17	96	97
4	1	12	13	10	99	96
5	6	3	12	11	97	93
Mean ± SD	4.8 ± 3.1	8.6 ± 3.4	12.6 ± 2.3	12.6 ± 2.7	96.4 ± 2.2	95.0 ± 1.9

[a] Determined by Wright's staining.
[b] Representative of all bead-ingesting phagocytes.
[c] Ratio of all bead-ingesting phagocytes which are stained with CM277.
[d] Ratio of all CM277-stained cells which contain phagocytized beads.

± 2.2% of bead-ingesting cells were stained by MoAb CM277 and that 95.0 ± 1.9% of the CM277-stained cells contained phagocytized beads. Figure 2 is a photomicrograph of the CM277-stained phagocytes containing internalized latex beads.

The binding of MoAb CM277 to monocytes was also shown by correlating this marker with HE-rosette formation. PBL were treated with MoAb CM277, mixed with HE, and the HE-rosetting cells quantitated by immunofluorescence for the presence of the CM277 marker. As shown in Table II, virtually all of the HE-rosetting monocytes (94.6 ± 4.5%) were labeled by CM277, and most of the cells labeled by CM277 formed rosettes with HE (94.0 ± 1.7%). The results indicate that the majority of the CM277-positive cells in the peripheral blood were monocytes. Figure 3 is photomicrographs of HE-rosetting monocytes which have phagocytized latex beads and are labeled with MoAb CM277. We confirmed that MoAb CM277 was binding to peripheral blood PMN by staining purified populations of these cells with the antibody and examining them by fluorescent microscopy. All PMN were found to be positively stained by CM277 (data not shown). The binding specificities were also confirmed using cytofluorometry. Only bitmaps gated for monocytes and PMN registered cells which were positively stained by CM277 (data not shown). The above observations indicate that MoAb CM277 is an effective marker for both monocytes and PMN in feline peripheral blood.

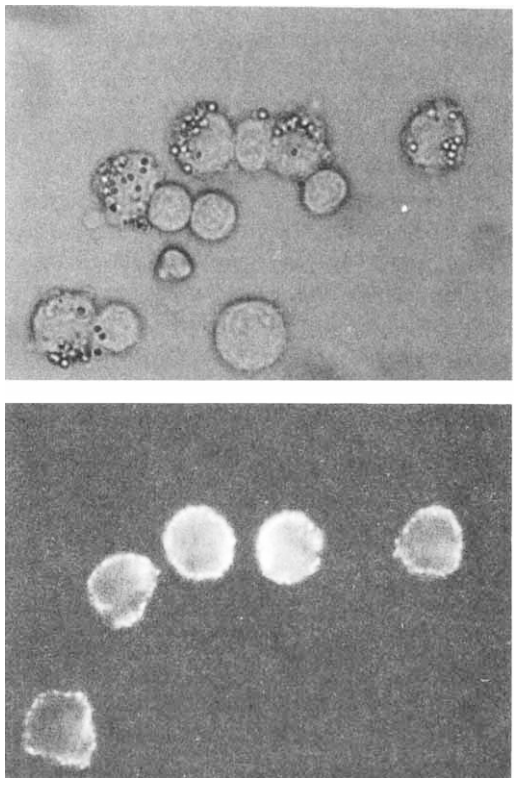

Figure 2. Photomicrographs of bead-ingesting phagocytes stained with the monoclonal antibody CM277 by indirect immunofluorescence.

Role of Monocytes and PMN in GPE-Rosette Formation. The nonspecificity of the guinea pig erythrocyte as a marker for feline peripheral blood T lymphocytes has been noted above. The apparent uncertainty concerning the contribution of PMN and monocytes to GPE-rosette formation justified a more detailed study. We used MoAb CM277 to differentiate PMN and monocytes from lymphocytes during GPE-rosetting assays. In fresh (Day 0) PBL it was found that practically all CM277-positive cells formed rosettes with GPE (96.7 ± 1.2%, Table III), indicating that the majority of PMN and monocytes in fresh PBL had GPE-rosetting capability. When only GPE-rosetting cells were examined for the presence of the CM277 marker, we found that an average of 59.0 ± 14.5% were CM277 positive (Table III). Thus, in fresh PBL, PMN and monocytes comprised a majority, but not all, of the GPE-rosettes. We assume that T lymphocytes comprise the remaining approximately 40% of the GPE-rosetting cells not accounted for by PMN and monocytes. Based on this analysis it may be estimated that

Table II. Correlation of MoAb CM277-Stained Cells with Those Which Form Rosettes with HE

Experiment	HE rosettes (%)	CM277 + (%)	PMN[a] (%)	CM277 + HE[b] / Total CM277 (%)	CM277 + HE[c] / Total HE (%)
1	5	6	3	95	100
2	14	25	1	95	97
3	22	17	4	94	91
4	17	21	1	91	96
5	17	14	1	95	89
Mean ± SD	15.0 ± 6.3	16.6 ± 7.2	2.0 ± 1.4	94.0 ± 1.7	94.6 ± 4.5

[a] Determined by Wright's staining.
[b] Radio of CM277-stained cells which form HE-rosettes.
[c] Ratio of HE-rosetting cells which are stained by CM277.

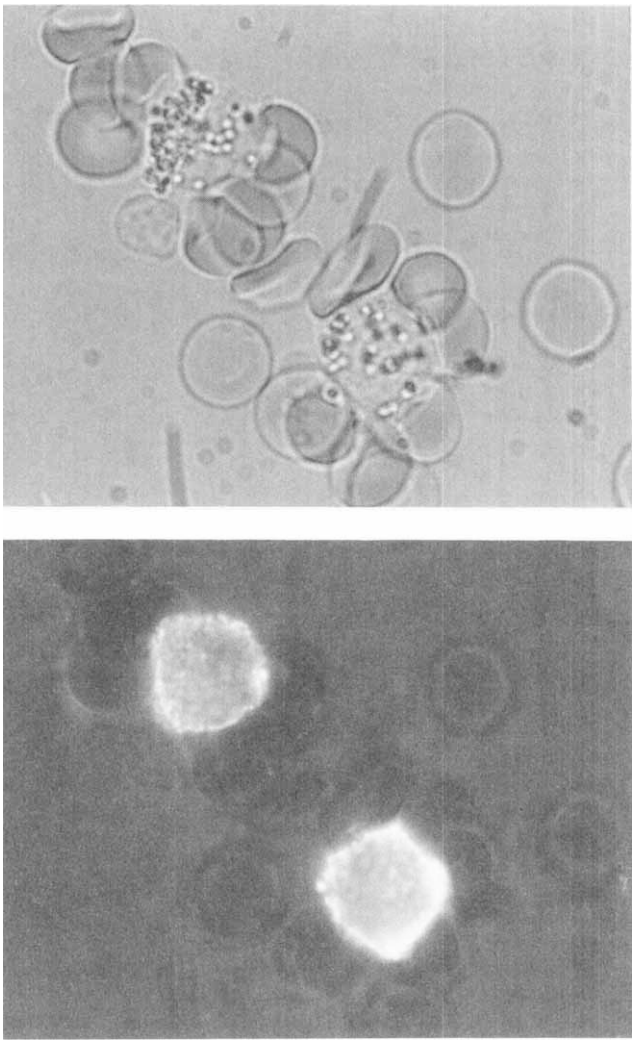

Figure 3. Photomicrographs of HE-rosetting cells which have phagocytized latex beads and are stained with the monoclonal antibody CM277 by indirect immunofluorescence.

in fresh PBL, 10 ± 5% of the cells are true GPE-rosetting T lymphocytes.

When feline PBL are cultured overnight at 37°C, there is an increase in the percentage of cells which can form rosettes with GPE. Like GPE-rosetting cells found in fresh PBL on Day 0, the GPE-rosetting cells found in Day 1 cultured PBL also produce IL-2 and facilitate the development of Ig-secreting B lymphocytes (10, 14). Given these properties, the newly arising GPE-rosetting cells are believed to be a subpopulation of T lymphocytes, and not monocytes or PMN. Our analyses of Day 1 PBL with GPE-rosetting and the CM277 marker support this conclusion. Thus, as shown in Table III there was a substantial *decrease* in the proportion of GPE-rosettes formed by PMN and monocytes (CM277-positive cells) after culturing, from 59.0 ± 14.5% in fresh PBL to 16.0 ± 3.6% in Day 1 PBL. This decrease could not be attributed to PMN and monocytes losing their receptors for GPE, since after culturing virtually all CM277-positive cells still formed rosettes with GPE (94.3 ± 1.5%, Table III). The *increase* of GPE-rosetting cells observed, therefore, after overnight culturing (18.7 ± 15.5% to 40.3 ± 5.7%, Table III) cannot be attributed to PMN and monocytes but in all probability, based on our functional studies (10, 14), reflect lymphocytes which develop GPE-rosetting capabilities. It should be noted that the changes observed in the percentage of GPE-rosetting cells are relative, i.e., extensive quantitative cell analyses of our culture system have revealed no increase in the absolute cell numbers, indicating that the percentage increase of GPE-rosetting cells represents a qualitative alteration in the cell population.

Effect of Monocyte Depletions from PBL on Mitogen-Induced Synthesis of Ig and IL-2. Our previous studies indicated that GPE-rosetting T lymphocytes are responsible for providing a helper activity necessary for the development of IgG-secreting B lymphocytes (6, 10). This conclusion was based on the observation that the depletion of GPE-rosetting cells from PBL on Day 0 or Day 1 caused a marked decrease in the development of IgG-secreting PFC as measured by the PWM-induced RHPA. The reduction in PFC was attributed to a loss of helper activity provided by the depleted GPE-rosetting T lymphocytes. However, because monocytes were also observed to form rosettes with

GPE, it was questioned whether these cells were responsible for facilitating the development of PFC. We examined the accessory role of monocytes in the formation of PFC by depleting these cells from PBL using HE-rosetting. As shown in Table IV, there were no consistent changes in PFC development corresponding to the depletion of monocytes ($P > 0.20$). The populations of PMN, GPE-rosetting T lymphocytes, B lymphocytes ($P > 0.5$), and GE-rosetting T lymphocytes ($P < 0.1 > 0.05$) in the PBL samples were not significantly altered following the HE-rosette depletions (data not shown).

We next determined whether the HE-rosetting monocytes played a role in another functional immune assay with PBL, that of IL-2 production. Both Day 0 and Day 1 PBL were examined since our previous studies had shown that the latter cells produced significantly more IL-2 than the fresh Day 0 cells (10, 14). The results of the leukocyte/E-rosette depletions of PBL with HE and GPE are shown in Table V. With Day 0 cells, removal of the HE-rosetting cells led to a significant increase of IL-2 synthesis over the control PBL ($P < 0.02$), while the GPE-rosette depletion lowered the amount of IL-2 produced ($P < 0.05$). With the Day 1 cells, there was a marked increase of IL-2 production by the control PBL over the Day 0 cells, in agreement with our previous study. Following the HE-rosette depletion of Day 1 PBL, the synthesis of IL-2 was increased, but not significantly, over the control ($P < 0.1 > 0.05$); this is in marked contrast to the effects of the GPE-rosette depletions, where the amount of IL-2 produced by the control Day 1 PBL was reduced from a mean of 24 units/ml to 4 units/ml after removal of the GPE-rosetting cells ($P < 0.01$). The effectiveness of the HE- and GPE-rosette depletions for each leukocyte/E-rosetting species is indicated in the footnotes of Table V, i.e., less than 1% of the specific erythrocyte-rosetting cells were left behind in the Day 0 PBL, and less than 2% of GPE-rosetting cells in the Day 1 PBL. Attention is drawn to the *spontaneous* loss of monocytes, the HE-rosetting cells, in PBL cultured overnight. In Day 0 PBL, the percentage of these cells was 7.6 ± 1.49, while in Day 1 PBL, the percentage had fallen to 1.26 ± 0.74. The increased percentage of GPE-rosetting cells in Day 1 PBL over that of the Day 0 PBL is also indicated, going from a mean of 33% on Day 0 to a mean of 51% in Day 1 PBL. This increase corresponds to our previous studies (6, 10) and also to the data shown in Table III.

Discussion

The data presented in this study have revealed that feline monocytes are capable of rosetting with HE. We have yet to determine whether tissue macrophages also rosette with HE. The work of Lalezari *et al.* (15) suggests this may be true. They reported that alveolar macrophages of the cat formed rosettes with HE, GPE, and canine and rat erythrocytes. Studies of HE-rosetting with other feline tissues have not been published. Although Cockerell *et al.* (3) also tested feline PBL for rosette formation with HE, no HE-rosetting cells were reported. It is possible that these investigators had a few monocytes in their PBL preparations, or alternatively, that the HE-rosettes were overlooked.

We found HE-rosette formation to be a valuable tool for depleting monocytes from peripheral blood. HE-rosetting followed by density gradient centrifugation has been much more effective than methods requiring adherence, such as incubations on plastic and the use of Sephadex G-10, neither of which have been

Table III. Correlation of MoAb CM277-Stained Cells (Monocytes and PMN) with GPE-Rosette Formation in Fresh PBL (Day 0) and PBL Cultured *In Vitro* Overnight (Day 1)

Experiment		CM277 + (%)	GPE rosettes (%)	CM277 + GPE[a] / Total GPE (%)	CM277 + GPE[b] / Total CM277 (%)
1					
Day 0[c]		2	14	44	98
Day 1[d]		0	34	17	94
2					
Day 0		17	36	73	96
Day 1		9	45	12	96
3					
Day 0		2	6	60	96
Day 1		1	42	19	93
Mean ± SD	Day 0	7.0 ± 8.7	18.7 ± 15.5	59.0 ± 14.5	96.7 ± 1.2
	Day 1	3.3 ± 4.9	40.3 ± 5.7	16.0 ± 3.6	94.3 ± 1.5

[a] Ratio of all GPE-rosetting cells which are stained by CM277.
[b] Ratio of all CM277-stained cells which form GPE-rosettes.
[c] Percentages determined in fresh PBL.
[d] Percentages determined in PBL which were cultured *in vitro* overnight (approximately 18 hr).

Table IV. RHPA after Depletion of HE-Rosetting Monocytes from PBL

Experiment	Plaque-forming cells/10^6 cultured cells[a]	
	Control PBL	HE-rosette depleted
1	401 (2)[b]	367 (0)
2	705 (3)	861 (0)
3	319 (9)	281 (1)
4	528 (2)	383 (1)
5	178 (16)	450 (0)
6	249 (9)	114 (0)
Mean ± SD[c]	397 ± 194 (6.4 ± 5.5)	409 ± 250 (0.3 ± 0.5)

[a] Mean of triplicate cultures having <15% variation of plaque-forming cells between cultures.
[b] Percentage of HE-rosetting cells in parentheses.
[c] HE rosette-depleted values were not significantly different from controls ($P > 0.5$) as determined by Student's t test for dependent samples.

Table V. IL-2 Production by Cat PBL following Depletion of HE- or GPE-Rosetting Cells

Experiment	Day 0 cells,[a] rosette depletions[c]			Day 1 cells,[b] rosette depletions[d]		
	Control	HE	GPE	Control	HE	GPE
1	14.7[e]	21	10	35	45	8
2	4	6	1.5	18.4	16	3
3	5.6	11	3.5	22.6	24	3.4
4	2.3	6.7	1.5	22.6	32	2.8
Mean ± SE	6.65 ± 2.35	11.18 ± 3.05	4.13 ± 1.74	24.65 ± 3.10	29.24 ± 5.34	4.3 ± 1.07

[a] Day 0 cells = cells stimulated with concanavalin A on day obtained from animal.
[b] Day 1 cells = cells that had been in culture for 1 day at 37°C before stimulation with concanvalin A.
[c] Percentage of HE rosettes: control PBL, 7.6 ± 1.49; after depletion, 0.24 ± 0.09. Percentage of GPE rosettes: control PBL, 33.25 ± 5.14; after depletion, 0.7 ± 0.18.
[d] Percentage of HE rosettes: control PBL, 1.26 ± 0.74; after depletion 0. Percentage of GPE rosettes: control PBL, 51.25 ± 3.93; after depletion, 1.87 ± 0.32.
[e] IL-2 units as described in Materials and Methods.

very successful in our laboratory. This inability to manipulate monocytes in our earlier experiments resulted in an uncertainty concerning their role in PWM-induced Ig synthesis. We had shown that the depletion of GPE-rosetting cells from PBL consistently leads to a substantial decrease (>80%) in the development of PFC (6, 10). This decrease was attributed to the elimination of GPE-rosetting T cells which were providing a helper activity. Since, however, monocytes and PMN were also known to form rosettes with GPE (7), we questioned whether the removal of monocytes and not T lymphocytes was the cause of the lowered response. In the present study, the removal of monocytes from PBL by HE-rosetting had no effect on the generation of PFC as measured in the RHPA. This finding supports our earlier interpretation that the decrease in PFC caused by GPE-rosette depletions is due to the loss of a population of T helper lymphocytes, not monocytes. Additional evidence for a T helper component in the GPE-rosetting population was demonstrated by the marked decrease in IL-2 production following their removal from PBL, confirming our original studies on a positive contribution of these cells in the synthesis of this lymphokine (10) (Table V).

Although neither a negative nor positive role could be attributed to monocytes in the RHPA assay, an immunobiologic function of these cells in IL-2 production was revealed. Thus, HE-rosette depletions of Day 0 PBL led to an enhanced synthesis of IL-2. Increased IL-2 formation by human PBL following removal of most adherent cells has also been reported (16–18). The lack of a similar effect with Day 1 cells may be explained by the already enhanced IL-2 response by these cells, precluding the detection of the consequences of monocyte depletion. Indeed, the spontaneous loss of monocytes following the overnight culture of PBL may have contributed to the increased IL-2 production by Day 1 PBL. An increase in IL-2 production by human cells cultured overnight has been reported by Chouaib and Fradelizi (19) and was attributed to the loss of monocytes. Whether, however, the increase we have observed in IL-2 synthesis with Day 1 feline PBL can be explained simply by a decrease in monocytes is doubtful. Thus, while HE-rosette depletions of Day 0 cells resulted in a residual population of only 0.25% HE-rosetting cells in the nonrosetted fraction, the IL-2 response by these cells was much less than that obtained with Day 1 cells (11.18 units/ml vs 24.65 units/ml),

where the mean percentage of remaining HE-rosetting cells was 1.26% (Table V). Monocyte-associated factors controlling the IL-2 response include interleukin 1, found to be necessary for the activation of T cells by lectins, and prostaglandins (prostaglandin E_2), which have been shown to down-regulate IL-2 synthesis (17, 18, 20–23). The control mechanisms of interleukin 1 and prostaglandins on IL-2 production by feline T cells are yet to be investigated, but with respect to the present study it may be important to note that when human PBL are incubated overnight at 37°C, they are much less sensitive to the inhibitory effects of prostaglandins as measured by [^{3}H]thymidine incorporation into lectin-stimulated cells (24). The loss, therefore, of monocytes combined with any decrease in sensitivity of feline leukocytes to prostaglandins may explain the greater IL-2 response by the Day 1 cells.

In addition to demonstrating HE-rosette formation with feline monocytes, we developed a MoAb, CM277, with a binding specificity for feline peripheral blood monocytes and PMN. The surface antigen on feline monocytes and PMN bound by MoAb CM277 is unknown. Since both CM277 and HE can simultaneously interact with monocytes, it is unlikely that the HE receptor on monocytes forms the epitope bound by CM277; CM277 does not block HE-rosette formation. A literature survey of the reactivity of monoclonal antibodies with cells of other mammalian species revealed that CM277 has a binding pattern similar to that of antibodies which bind the Mac-1 (Mol or OKM1) antigen (CD11b/CD18). This antigen is present on human and murine monocytes, PMN, and natural killer cells (25–28). Studies of the composition and molecular weight of the antigen bound by CM277 are in progress and should reveal if CM277 is binding to the feline homologue of the human and murine CD11b/CD18 molecular complex.

The demonstrated cell specificity of MoAb CM277 permitted resolution of a primary objective of this study, i.e., a clarification of the contribution of monocytes, PMN, and T lymphocytes to the GPE-rosetting population. In agreement with our earlier observations, we conclude that monocytes, when present, can comprise a significant proportion of GPE-rosetting cells in fresh PBL. Many of these cells, however, are lost upon overnight incubation and form only a small proportion of the GPE-rosetting cells on Day 1, while GPE-rosetting T cells predominate. Thus, based on our data in Table III, we estimate the T lymphocytes comprise about 65–75% of the GPE-rosetting population after PBL are cultured overnight, and therefore, 25–35% of all PBL. When included with the 10% GPE-rosetting T lymphocytes calculated to exist in fresh PBL (Table III), the entire GPE-rosetting T lymphocytes population forms approximately 35–45% of the cells in the peripheral blood of the cat. Both populations of GPE-rosetting T cells, those present on Day 0 and those appearing on Day 1, have been shown to exert a T helper function in the mitogen-induced synthesis of Ig and IL-2 (10, 14).

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