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Further evidence that antibody-dependent and spontaneous cell-mediated cytotoxicity are mediated by different processes or cell types

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Summary. We have observed that canine peripheral blood mononuclear cells are ineffective as mediators of spontaneous cell-mediated cytotoxicity (SCMC) despite being excellent mediators of antibody-dependent cellular cytotoxicity (ADCC). Canine lymphocytes were unable to kill seven cell lines in SCMC assays including Chang, K-562, dog kidney, and foetal intestine. One the other hand, they were able to kill Chang and K-562 cell line cells as well as chicken red blood cells in ADCC assay systems. Canine mononuclear cells were 40% E-rosette forming, 30% surface immunoglobulin bearing, 14% Fc receptor bearing, and 13% esterase staining. K-562 cell line cells inhibited the capability of human peripheral blood cells but not canine peripheral blood cells to kill CRBCs in an ADCC assay. Fc-receptor bearing human lymphocytes of both T-cell and null-cell subclasses mediated both SCMC and ADCC, while Fc-receptor bearing canine lymphocytes mediated ADCC but not SCMC. These observations add to the evidence of a dichotomy between SCMC and ADDC with regards to either cell type or cell processes.

* The authors deeply regret the death of Dr Joseph F. Formeister on February 7, 1979. His untimely passing was a personal and scientific loss deeply felt by the authors, Dr Formeister's surgical colleagues, and all who knew him as a dedicated physician and researcher.

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INTRODUCTION

Spontaneous cell-mediated cytotoxicity (SCMC) and antibody-dependent cellular cytotoxicity (ADCC) are both well established *in vitro* systems which may reflect *in vivo* cytotoxic processes involved in tumour rejection, graft rejection, host defence mechanisms, and autoimmune diseases. Both phenomena appear to be mediated by Fc-receptor bearing lymphocytes termed K cells for ADCC (Pape, Troye & Perlmann, 1977; Nelson, Bundy, Pitchon, Blaese & Strober, 1976; Brier, Chess & Schlossman, 1975) and NK cells for SCMC (Kiessling, Klein, Pross & Wigzell, 1975; Kiessling, Petranyi, Kärre, Jondal, Tracey & Wigzell, 1976; Peter, Pavie-Fischer, Fridman, Aubert, Cesarini, Roubin & Kourilsky, 1975). It has not been totally clear, however, as to whether K and NK lymphocytes are identical cells and whether identical cell receptors or cell processes mediate both SCMC and

ADCC. Recent observations have disclosed differential effects of trypsin pre-treatment (Kiessling *et al.*, 1976; Peter *et al.*, 1975; Kay, Bonnard, West & Herberman, 1977; Nelson, Bundy & Strober, 1977), aggregated immunoglobulins (Kiessling *et al.*, 1976), staphylococcal protein A (Kay *et al.*, 1977), and simple sugars (MacDermott, Kienker & Muchmore, 1980) on ADCC and SCMC. Additional evidence of a dichotomy between SCMC and ADCC has come from observations in immunodeficient patients (Koren, Amos & Buckley, 1978a), and foetal piglets (Koren, Amos & Kim 1978b) showing one or the other cytotoxic capability alone.

The present study reports our observations using mononuclear cells from the peripheral blood of canines. We have found that adult canine peripheral blood mononuclear cells are unable to mediate SCMC and yet mediate ADCC with ease.

MATERIALS AND METHODS

Isolation of effector lymphocytes

Sixteen healthy mongrel dogs were selected for study. Eight animals were female and eight were male. Peripheral blood samples were collected in a heparinized syringe and isolated by Ficoll–Hypaque centrifugation as described by Böyum (1968). Similarly, human peripheral blood mononuclear cells were isolated from healthy volunteers by Ficoll–Hypaque centrifugation (Böyum, 1968). The cells from interface layers were washed three times in M-199 assay medium: medium 199 (Microbiological Associates, Bethesda, MD), 2% 200 mM L-glutamine, 1% 1 M HEPES buffer, 5% heat-inactivated foetal calf serum, 1% penicillin–streptomycin solution (5000 units–5000 μg/ml) (GIBCO, Grand Island, NY), 0·5% 1 M $CaCl_2$, and NaOH (Sigma Chemical Co, St Louis, MO) to bring the pH to 7·4, counted and viability determined (routinely > 95% for both canine and human cells) by trypan blue exclusion.

Cell lines

Chang and K-562 cell line cells were obtained from Drs John Ortaldo, David Kay, and Guy Bonnard of the National Cancer Institute and maintained as continuous cell lines. HeLa and Raji cell line cells were obtained as needed from Drs Alison O'Brien and Carter Diggs. Dog kidney (NBL-2), human foetal intestine (407) and mouse NCTC cell line cells were obtained from the American Type Culture Collection, Rockville, MD.

Cytotoxicity studies

The cytotoxic capabilities of canine and human peripheral blood lymphocytes were assessed in the following assays: antibody-dependent cellular cytotoxicity (ADCC) against chicken RBCs; lectin-induced cellular cytotoxicity (LICC) against human RBCs (MacDermott, Nash, Saint, Clark, Zaras & Boldt, 1976); Chang ADCC and spontaneous cell-mediated cytotoxicity (SCMC); K-562 SCMC and ADCC; and SCMC against HeLa, dog kidney, foetal intestine, Raji, and mouse NCTC cell line cells.

Preparation of ^{51}Cr-labelled chicken or human red cell targets

One millilitre each of chicken or allogeneic human blood from normal volunteers was drawn in a heparinized syringe and washed three times in M-199 assay medium. After washing, 15 μl of the resulting pellets were mixed with 25 μCi of [^{51}Cr]-sodium chromate (200–500 Ci/g at 5 mCi/ml, New England Nuclear, Boston, MA) in separate tubes and incubated for 2 h at 37° with resuspension every 15 min. The ^{51}Cr-labelled RBCs were then washed three times in M-199 assay medium, resuspended, counted, and diluted to a final concentration of 2×10^5 cells/ml.

Preparation of ^{51}Cr-labelled cell line targets

Chang, HeLa, dog kidney, foetal intestine, Raji, and mouse NCTC cell line cells were harvested with a rubber policeman and decanted with their culture medium (Eagle's Minimum Essential Medium); K-562 cells, cultured in suspension, were decanted; and all cell line cells were spun individually at 400 g for 10 min. The supernates were decanted and 25 μCi ^{51}Cr were added directly to each pellet. The cells were then incubated and washed in the same manner as the RBC targets and diluted in M-199 assay medium to a final concentratration of 1×10^5 cells/ml.

Assays

Assays were performed in triplicate in round-bottomed microtitre plates. For ADCC assays, 50 μl per well of rabbit anti-chicken RBC, anti K-562, or anti-Chang cell antibody was used to coat 50 μl of the final suspension of the respective ^{51}Cr-labelled

target cells. Appropriate dilutions of effector lymphocytes in M-199 assay medium were added in 50 μl aliquots to yield effector to target (E:T) ratios of 100:1, 50:1, and 10:1. For each E:T ratio, 50 μl of M-199 assay medium was substituted for antibody as a control. SCMC against ^{51}Cr-labelled cell line cells was performed in a manner similar to the ADCC assays, except that no antibody was used.

LICC against human ^{51}Cr-labelled RBCs was carried out using the same techniques as for chick RBC-ADCC except that wheat germ agglutinin (WGA) (Miles-Yeda Ltd) at a concentration of 5 μg/ml was used instead of antibody. At this concentration, WGA did not cause cytotoxicity by itself.

After incubation at 37° for 4 h for chick ADCC or 16 h for the other assays, the plates were harvested using a Titertek harvesting system (Flow Labs) and the amount of ^{51}Cr label in each supernatant (counts per minute, c.p.m.) determined in a Beckman BioGamma gamma counter. Triplicate c.p.m. values usually varied less than 5% from one another. Parallel incubation mixtures containing radiolabelled RBC or cell lines in media alone were used to determine spontaneous ^{51}Cr release. Maximal ^{51}Cr release was determined in additional incubation mixtures containing only radiolabelled RBC (or cell lines) which had been disrupted by detergent lysis. Maximal ^{51}Cr release averaged 90%, spontaneous release with cell line targets approximately 25% and spontaneous release with red cell targets approximately 10% of the incorporated chromium. The spontaneous release without lectin or antibody but with effector white blood cells (WBC) was essentially the same as that without WBC. Using the average value of triplicate release values, the percentage cytotoxicity was determined by the formula:

$$\% \text{ cytotoxicity} = \frac{\text{Experimental release (c.p.m.)} - \text{spontaneous release (c.p.m.)}}{\text{Maximal release (c.p.m.)} - \text{spontaneous release (c.p.m.)}} \times 100$$

Surface characteristics of cell populations

Mononuclear cells from canines capable of forming spontaneous rosettes with freshly obtained human red blood cells were assayed using a modification of the technique described by Zander, Boopalam & Epstein (1975). Mononuclear cells from humans capable of forming rosettes with sheep red blood cells (Colorado Serum Co, Denver, CO) were assayed using similar methods. In brief, 2×10^5 canine or human mononuclear cells and 20×10^6 human or sheep red blood cells respectively were combined in 0·2 ml of foetal calf serum which had been absorbed with human or sheep red blood cells. The cells were incubated for 18 h at 4°. The cells were resuspended and 100 lymphocytes counted to determine the percentage of E-rosette forming cells. A lymphocyte with three or more RBCs attached was considered a rosette. Slight modifications of the technique of Ehlenburger & Nussenzweig (1976) were used to prepare EA or EAC cells. Sheep red blood cells were washed four times in RPMI 1640 and resuspended to 5% (v/v) in HBSS. EA cells were prepared by adding 7s IgG rabbit anti-SRBC (Cordis Labs, Miami, Florida) to concentrations of 1:100 to 1:6400 while EAC cells utilized 19S IgM rabbit anti-SRBC (Cordis Labs). After 1 h incubation at 37°, EA cells were centrifuged at 400 ***g*** for 5 min, resuspended and examined for agglutination. Fresh human serum was incubated with the 19S EA cells to obtain EAC cells. The cells with the highest non-agglutinating concentration of antibody were washed three times and resuspended to 0·5% (v/v) in HBSS plus 0·02% sodium azide. For EA or EAC rosette determination, 5×10^5 separated canine or human mononuclear cells in 0·25 ml HBSS plus 0·2% sodium azide were added to an equal volume of indicator red blood cells, centrifuged for 5 min at 50 ***g*** and incubated at 37° for 30 min. Smears were prepared in foetal calf serum and rosettes counted using 0·1% toluidine blue stain.

A direct fluoresceinated antibody technique employing fluoresceinated goat $F(ab')_2$ anti-human $F(ab')_2$ was used to detect surface immunoglobulin on human cells (Chess, MacDermott & Schlossman, 1974). Separated mononuclear cells (2×10^6) were resuspended in 0·02 ml of fluorescein-conjugated goat $F(ab')_2$ anti-human $F(ab')_2$ (IgG + IgD + IgM + IgA) (Kallestad, Chaska, NM). Cells were incubated for 30 min at 37° with one resuspension every 5 min. Cells were washed three times in medium 199 plus 10% foetal calf serum and resuspended in two drops of PBS-glycerine. The slides were observed on a Zeiss Photomicroscope III. The percentage of canine mononuclear cells bearing dog surface immunoglobulins was determined using the same techniques except that the fluoresceinated reagents employed were: (1) fluorescein-conjugated IgG fraction, goat anti-dog IgM (heavy chain), lot 7439, Cappel

Laboratories, Cochranville, PA, 19330; and (2) fluorescein-conjugated IgG fraction rabbit anti-dog IgG (heavy and light chains), lot 9717, Cappel Laboratories. Monocytes were defined by use of the non-specific esterase stain employing α-naphthyl butyrate (Sigma Chemical Co, St Louis, MO) as substrate (Koski, Poplack & Blaese, 1976).

Studies on Inhibition by trypsin or cold targets

Effector mononuclear cells were incubated with 0·5% trypsin (A grade, Sigma Chemical Co., St Louis, MO) in Hanks's balanced salt solution (GIBCO), for 15 min at 37°. The cells were then washed four times in M-199 assay medium, resuspended, counted, viability determined (routinely greater than 90%), and used as effector cells in the cytotoxicity assays. The ability of unlabelled K-562 cell line cells to inhibit killing of chromium labelled chicken red blood cells in an ADCC assay was determined by adding varying numbers of K-562 cells to the ADCC assay. The number of unlabelled K-562 cells ranged from none to 100 in relation to each labelled CRBC.

Cell separation techniques

Human leucocyte subpopulations were obtained using the methods described by Chess *et al.* (1974) and modified as described in part by Broder, Edelson, Lutzner, Nelson, MacDermott, Durm, Goldman, Meade & Waldman (1976), and Nelson & MacDermott (1977). In brief, peripheral blood mononuclear cells ingesting iron particles were removed by magnetic field depletion and density gradient sedimentation over Ficoll–Hypaque (1200 ***g*** for 5 min). Monocyte depleted lymphocytes were incubated and then passed over a Sephadex-G-200 anti-human Fab immunoabsorbent column to separate the cells into a non-immunoglobulin bearing (T-cell enriched) cell population and an immunoglobulin bearing (B-cell enriched) cell population. The T-cell enriched population was fractionated using overnight sheep red blood cell (E) rosette formation followed by Ficoll–Hypaque density sedimentation. The non-E-rosette forming lymphocytes (null cells) at the interface were then harvested. T cells were further purified by distilled water lysis of the E-rosetted non-immunoglobulin-bearing cells and then Fc-receptor bearing T cells or contaminating null cells were removed by formation of rosettes with chicken RBCs (CRBC) coated with anti-CRBC antibodies (Cappel Labs, Cochranville, PA) followed by Ficoll Hypaque density sedimentation. T cells in the B-cell population were removed by formation of overnight E rosettes (at 4°) followed by Ficoll–Hypaque sedimentation. Macrophages were obtained by incubating peripheral blood mononuclear cells overnight in plastic petri dishes and harvesting the adherent cells. Granulocytes were harvested by dextran sedimentation as described by Böyum (1968) from the pellet of the Ficoll–Hypaque separation used to isolate blood mononuclear cells. Canine peripheral blood leucocyte subpopulations were obtained using the iron carbonyl technique described above to deplete macrophages and the CRBC-EA-rosette forming technique described above to deplete and enrich Fc-receptor bearing cells.

Surface characteristics of separated cells

In the cytotoxic assays performed in this study, the surface characteristics of the separated human leucocyte subpopulations were: WBC (54% E-rosette forming (E+), 13% surface-immunoglobulin bearing (SIg+), 9% EA-rosette forming (EA+), and 18% esterase staining (Est+); T + null (61% E+, <1% SIg+, 10% EA+, <1% Est+); T (E+) (86% E+, <1% SIg+, 11% EA+, <1% Est+); T(E+, EA−) (94% E+, <1% SIg+, 2% EA+, <1% Est+); T (E+, EA+) (86% E+, <1% SIg+, 65% EA+, <1% Est+); B (3% E+, 93% SIg+, 16% EA+, <1% Est+); null (4% E+, <1% SIg+, 61% EA+, <1% Est+); and macrophages (10% E+, 14% SIg+, 54% EA+, 85% Est+). The surface characteristics of the separated canine subpopulations were: WBC (see Table 3); lymphocytes (16% EA+, <1% Est+); EA−, lymphs (2% EA+, <1% Est+); EA+ lymphs (72% EA+, <1% Est+); and macrophages (41% EA+, 84% Est+).

RESULTS

Cytotoxic capabilities of canine peripheral blood mononuclear cells

Our initial experiments, done at 3 killer to target cell ratios, are shown in Table 1. Mononuclear cells from six to eight different adult healthy dogs failed to mediate SCMC of either Chang or K-562 cell line cells yet mediated excellent LICC of HRBC and ADCC of CRBC, Chang, and K-562 targets. In contrast, mononuclear cells from four to six different healthy humans worked well in all assays. In order

Table 1. Cytotoxic capabilities of human and canine peripheral blood mononuclear cells in LICC, SCMC and ADCC assays

Effector cells		Cytotoxicity (%) in: HRBC LICC	CRBC ADCC	Chang SCMC	Chang ADCC	K-562 SCMC	K-562 ADCC
Human	100:1	63±8 (4)*	69±8 (6)	32±12 (4)	50±10 (4)	44±10 (5)	62±3 (5)
	50:1	61±7 (4)	54±5 (6)	12±3 (4)	35±9 (4)	43±8 (5)	63±8 (5)
	10:1	59±4 (4)	41±8 (6)	3±1 (4)	18±6 (4)	29±6 (5)	52±6 (5)
Canine	100:1	84±9 (6)	83±9 (8)	1±0·5 (6)	46±4 (6)	2±0·5 (7)	46±6 (7)
	50:1	80±6 (6)	68±8 (8)	1±0·3 (6)	38±6 (6)	1±0·6 (7)	20±0·8 (7)
	10:1	54±3 (6)	50±6 (8)	0·2±0·1 (6)	4±0·5 (6)	2±1 (7)	6±1 (7)

* Mean ± SEM, for the number of experiments shown in parentheses.

better to validate the apparent lack of SCMC capabilities exhibited by canine mononuclear cells, we used additional cell lines as targets. Mononuclear cells from three different healthy dogs did not mediate SCMC against HeLa, dog kidney, human foetal intestine, Raji, or mouse liver cell lines, whereas control cells from three humans mediated SCMC against three of the five cell lines (Table 2). In summary, canine mononuclear cells were effective in LICC and ADCC but were ineffective in the killing of seven cell lines by SCMC. Of particular note are the observations that a canine cell line was not killed in SCMC by canine mononuclear cells and that two of the cell lines not killed in SCMC (Chang and K-562) were killed in ADCC assays.

Surface characteristics of canine mononuclear cells

Because the question arises as to the nature of the cells being studied, we investigated surface characteristics of canine peripheral blood mononuclear cells. As seen in Table 3, 40% of cells formed E rosettes with human RBCs, 14% bore Fc receptors as detected by EA rosettes (SRBC; IgG and anti-SRBC), 31% bore complement receptors as detected by EAC rosettes (SRBC; IgM anti-SRBC and fresh human serum), 30% had surface IgG and 8% surface IgM as detected by fluoresceinated anti-dog IgG and IgM, respectively; and 13% stained with esterase. Thus, canine peripheral blood mononuclear cells appeared to contain all of the different types of mononuclear cell subclasses currently recognized and most importantly, for these studies, contained Fc-receptor bearing cells.

Inhibition experiments

We next carried out cold target inhibition experi-

Table 2. Comparison of human and canine peripheral blood mononuclear cells in SCMC of additional cell lines

Effector cells		Cytotoxicity (%) exhibited against the cell lines: HeLa	NBL-2 (Dog kidney)	Human foetal intestine	Raji	NCTC (Mouse liver)
Human	100:1*	18±3†	32±6	25±8	2±0·3	1±0·8
	50:1	17±4	28±1	23±2	0·3±0·5	0·6±0·2
	10:1	15±3	18±4	19±5	0·6±0·5	0·8±0·6
Dog —	100:1	2±0·7	0·8±0·6	3±1	0·4±0·1	0·7±0·2
	50:1	2±1	2±0·2	0·8±0·4	0·7±0·5	0·4±0·2
	10:1	0·7±0·2	0·8±0·4	1±0·8	0·2±0·2	1±0·5

* Effector to target cell ratio.

† Mean ± SEM for three experiments, each done in triplicate.

Table 3. Surface characteristics of canine peripheral blood mononuclear cells

E	*EA*	*EAC*	*SIgG*	*SIgM*	*Esterase*
40±7 (8)*	14±4 (6)	31±7 (6)	30±8 (6)	8±3 (6)	13±2 (11)

* Mean ± SEM of determinations, with the number of samples noted in parentheses.

Table 4. Cold target inhibition of CRBC ADCC by addition of unlabelled K-562

	Cytotoxicity (%) mediated by mononuclear cells from:	
Ratio K-562:CRBC	Humans	Canines
0:1	42±6*	44±7
1:1	41±8	43±9
10:1	20±5	41±2
100:1	5±2	40±5

* Mean ± SEM for three experiments, each done in triplicate at a 10:1 killer to target cell ratio.

ments using canine cells to see if a target which was not killed in SCMC (K-562) could interfere with killing of a target which was killed in ADCC (CRBC). As can be seen in Table 4, ADCC of CRBC by human mononuclear cells was inhibited if there were ten or more unlabelled K-562 cell line cells for each labelled CRBC. In contrast, ADCC of CRBC by canine mononuclear cells was not inhibited by K-562 cell line cells with up to 100 K-562 cells for each CRBC. Thus, canine effector cells were not capable of killing K-562 targets (Table 1), and these targets did not interfere with killing of CRBC (Table 4).

Effector cell types

Finally, we examined the effector cell types active in ADCC and SCMC assays using human and canine cells as effectors. As shown in Table 5, using standard separation techniques on human cells, we found that Fc-bearing T cells and Fc-bearing null cells were the principal mediators of ADCC and SCMC, whereas T cells not bearing Fc receptors and B cells were not effectors. Human macrophages and granulocytes were able to mediate ADCC with CRBC as targets but not with cell lines as targets. Using EA-rosette separation and carbonyl iron depletion techniques on canine mononuclear cells, we found that Fc-receptor bearing canine lymphocytes were capable of mediating ADCC whereas Fc-receptor negative lymphocytes could not. Neither subclass of canine lymphocytes were capable of mediating SCMC against the cell lines examined (Table 6). All

Table 5. Subclasses of human peripheral blood mononuclear cells capable of mediating SCMC, ADCC and LICC

	Cytotoxicity (%) in:			
Effector cell	CRBC ADCC	K-562 SCMC	K-562 ADCC	HRBC LICC
WBC	55±10*	30±4	48±6	61±8
T + Null	28±6	13±2	28±4	57±6
T (E+)	18±3	4±1	5±0·5	48±9
T (E+, EA−)	5±1	0·5±0	0·6±0·1	37±6
T (E+, EA+)	31±7	17±4	31±7	53±8
B	5±1	1±0	1±0·3	39±4
Null	62±6	35±8	51±6	62±10
Macrophage	58±8	6±1	8±4	5±6
Granulocytes	73±9	0·2±0	0·5±0·2	78±4

* Mean ± SEM for five experiments, each done in triplicate, at a 10:1 killer to target cell ratio.

Table 6. Subclasses of canine peripheral blood mononuclear cells in cytotoxic assays

Effector cell		Cytotoxicity (%) in: CRBC ADCC	K-562 SCMC	K-562 ADCC	HRBC LICC
WBC	50:1	37±5*	0·9±0·3	34±4	64±9
	25:1	30±6	0·2±0·1	36±3	63±7
Lymphocytes	50:1	45±5	0·2±0·1	40±7	69±4
	25:1	36±6	0·4±0·2	28±5	65±6
EA− lymphs	50:1	8±1	0·3±0·1	6±1	54±5
	25:1	3±0·5	0·2±0·1	4±0·6	58±6
EA+ lymphs	50:1	42±5	0·3±0·2	31±4	57±3
	25:1	41±5	0·4±0·1	29±5	52±9
Macrophage	50:1	35±6	0·2±0·1	1±0·3	62±5
	25:1	20±3	0·2±0·1	0·4±0·2	65±6

* Mean ± SEM for four experiments, each done in triplicate.

of the cell subpopulations from both humans (Table 5) and canines (Table 6), were effectors of LICC with HRBC as targets.

DISCUSSION

These studies have revealed that canine peripheral blood mononuclear cells are capable of mediating ADCC but not SCMC. This provides additional evidence that ADCC and SCMC are mediated either by two separate cell types or by two different processes within the same cell.

In previous studies describing SCMC, it has been noted that trypsin would inhibit SCMC but not ADCC. Peter *et al.* (1975) observed a slight decrease in SCMC but not ADCC using 0·25% trypsin pretreatment of effector cells for 30 min and Kiessling *et al.* (1976) observed a marked decrease in SCMC but no effect on ADCC using 0·25% or 1·0% trypsin for 45 min at 37°. These results were later confirmed by Kay *et al.* (1977) and Nelson *et al.* (1977). Additional information in this respect was provided by Kiessling *et al.* (1976) who observed that aggregated immunoglobulin would block ADCC and not SCMC, and by Kay *et al.* (1977) who observed that Staph protein A would inhibit ADCC and not SCMC. Both effects were presumably due to interference with Fc receptor and antibody interaction: aggregated immunoglobulins by binding to the Fc receptor and Staph protein A by binding to the Fc portion of immunoglobulins. Thus Fc receptors could be rendered non-functional without any effect on SCMC activity. Furthermore, a third differential effect has been noted by Trinchieri, Santoli & Koprowski (1978) with the observation that interferon and interferon inducers enhance SCMC but not ADCC activity. Finally, recent studies from our laboratory have revealed that a series of simple sugars [including D-(−)-ribose, β-gentiobiose N-acetyl-D-galactosamine, D-(+)-cellobiose, and α-lactose] at 25 mM or 50 mM concentrations will inhibit SCMC of either Chang or K-562 cell line cells but will not inhibit ADCC of either target cell type (MacDermott *et al.*, 1980).

In contrast to the above, investigations of the effector cell types capable of mediating SCMC and ADCC have uniformly disclosed that Fc-receptor bearing lymphocytes are the effector cell types in both systems. These cells are termed K cells in the ADCC system (Pape *et al.*, 1977) and NK cells in the SCMC system (Kiessling *et al.*, 1975). The findings of the present study with regards to human cells agree with those of others, although considerable controversy exists as to whether only Fc-receptor bearing T cells mediate ADCC and SCMC (Kay *et al.*, 1977; West, Cannon, Kay, Bonnard & Herberman, 1977) as opposed to K and NK cells in the null cell subclass of lymphocytes as well as Fc-receptor bearing T cells. Our studies indicate that despite extensive T-cell depletion, there still exist cells in the non-T, non-B cell subclass of lymphocytes which are effectors in both systems. Thus, we would feel that K and NK cell activity resides in both T and null cell subclasses. With regards to canine lymphocytes, Fc-

receptor bearing cells are present which mediate ADCC but not SCMC, indicatiing that factors in addition to the presence of an Fc receptor are involved in order to achieve SCMC.

A recent series of studies by Koren *et al.* (1978a, b, and c) has provided an approach to establishing differences between SCMC and ADCC activities which is similar to that used in the present study. Koren *et al.* (1978a) observed that patients with X-linked agammaglobulinaemia had normal SCMC, markedly impaired ADCC against cell line cells, and normal ADCC against CRBC targets. In contrast, patients with severe combined immunodeficiency had markedly impaired SCMC, normal ADCC with CRBC as targets and impaired ADCC with cell lines as targets. Thus, patients with immunodeficiency syndromes exhibited differences between SCMC and ADCC activities not seen with normal human peripheral blood mononuclear cells. In a subsequent study, Koren *et al.* (1978b) observed that adult Minnesota miniature pigs exhibited normal SCMC and ADCC capabilities. In contrast, these authors found that newborn piglets obtained 3 days prior to full term by hysterectomy exhibited normal ADCC but lacked SCMC. Thus, SCMC effector capability appeared to develop after birth in these animals, in contrast to ADCC which was present prior to full term. The results of our present study demonstrate an additional circumstance in which ADCC can be observed without SCMC activity. In contrast to previous observations, however, the cells which we used were not from either immunodeficient or foetal subjects. Thus, canines offer a healthy adult animal whose peripheral blood mononuclear cells are not mediators of SCMC yet excellent mediators of ADCC.

The types of cells present in canine peripheral blood have been described previously by Bowles, White & Lucas (1975) and Zander *et al.* (1975). Our results are in agreement with theirs with regards to E-rosette formation (39·8% by Bowles *et al.* (1975) and 38·4% by Zander *et al.* (1975). Examination of surface immunoglobulin resulted in identical results (30%) by ourselves and Zander *et al.* (1975), while Bowles *et al.* (1975) found 46·6% B cells using fluoresceinated anti-dog globulin. Our observation of only 8% of cells bearing IgM was not looked for previously. Likewise, there are no other data on the percentage of esterase positive cells, which we found to be 13%. Unfortunately, previous studies on EA and EAC rosettes gave quite varied results and used different reagents from ours. Using IgM reagents, Zander *et al.* (1975) found 23% and Bowles *et al.* (1975) 4% Fc-receptor bearing cells. We used an IgG EA reagent and noted 14% Fc-receptor bearing cells. Likewise, these previous studies using differing techniques noted 22–49·3% complement-receptor bearing cells while we found 31% EAC-rosette forming cells. Therefore, our observations indicate that approximately 40% of canine peripheral blood mononuclear cells are T cells, 30% are B cells, 15% are K cells, and 15% are macrophages.

An issue which the present study does not clarify is whether or not the difference between SCMC and ADCC is due to two different cell types or two different effector processes within the same cell. The recent study of Koren & Williams (1978c) presented evidence in favour of two different cells, a K cell with an Fc receptor and an NK cell with both an Fc receptor and another NK specific receptor. The basis of this conclusion was that SCMC could be inhibited by both SCMC sensitive targets and antibody-coated targets. On the other hand, ADCC was only inhibited by ADCC targets and not potential SCMC targets with the notable exception of K-562 which did inhibit ADCC. Koren & Williams (1978c) felt that the K-562 inhibition was due to Fc receptors on the K-562 cells which, by binding to the antibody-coated target, would prevent its destruction. Our results with human effector cells confirm their observation as to K-562 inhibition of ADCC. However, our results with canine effector cells would argue against their interpretation of their K-562 inhibition experiments in that K-562 targets had no effect on ADCC by canine effector cells and thus did not bind to the antibody-coated target to prevent lysis. Instead, it would appear that unlabelled K-562 cell line cells must be able to be killed in SCMC in order to inhibit ADCC. These observations in conjunction with our recent finding that simple sugars will block SCMC but not ADCC has led us to propose that NK specific receptors may be endogenous lectins (MacDermott *et al.*, 1980).

The present study, therefore, demonstrates a marked defect in SCMC capabilities in the peripheral blood mononuclear cells of a healthy adult animal despite normal ADCC activity. This defect could be due to any one of a number of possibilities. Canine peripheral blood mononuclear cells may contain only K cells and not NK cells. Another possibility is that NK specific receptors (which we feel may be endogenous lectins) on canine peripheral blood Fc-receptor

bearing cells could be absent. Alternatively, extracellular or intracellular processes that regulate cytotoxicity may not allow SCMC to occur despite the presence of NK cells or NK receptors. Finally, it should be noted that our findings are only related to canine peripheral blood mononuclear cells and do not rule out mediation of SCMC by canine cells from other lymphoid tissues. Investigation of canine mononuclear cells may allow further dissection of the differences between SCMC and ADCC and may also allow clarification of the *in vivo* functions of SCMC.

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