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RECOGNITION OF TWO EPITOPES OF AN ANTIGEN PRESENT ON CANINE T CELLS BUT NOT ON HEMOPOIETIC PROGENITORS BY FOUR MONOCLONAL ANTIBODIES[1]

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Pairs of murine monoclonal antibodies, which recognize 2 different epitopes on a single antigen are described. These antibodies (MdT-P_1, -P_2, -Q_1, -Q_2) defining a canine pan-T cell antigen, were raised against dog thymocytes. In immunoblotting of solubilized and polyacrylamide gradient gel electrophoresis in sodium dodecyl sulphate (SDS-PAGE) fractionated dog thymocytes, they revealed a strong heterogeneous antigen. Competitive inhibition of binding of directly labeled mouse-antidog T lymphocytes monoclonal antibodies (MdT-mAbs) to solubilized dog thymocytes indicates that 2 different antigenic epitopes (P, Q) are recognized. In indirect peroxidase immunocytochemistry, MdT monoclonal antibodies recognized up to 95% thymocytes, 69% blood lymphocytes, 76% lymph node lymphocytes, and approximately 2% bone marrow lymphocytes; they were nonreactive with surface immunoglobulin positive blood cells, monocytes, platelets, cells of myelo- and erythropoietic lineage in the bone marrow. Immunohistochemistry on thymus, lymph nodes, and spleen sections revealed that MdT-mAbs had labeled cortical and medullary thymocytes, paracortical T cell areas in lymph nodes and the periarteriolar zone of spleen white pulp, whereas B cell areas remained unstained. The antibodies lysed dog thymocytes in the presence of complement. Lethally irradiated dog receiving bone marrow autograft depleted of MdT-P_1 positive cells ex vivo showed engraftment and complete recovery of marrow function. Studies of antibody activity on canine hemopoietic progenitor cells in granulocyte-macrophage progenitors (CFU_{GM}) also showed no reduction of CFU_{GM} in MdT-P_1–depleted bone marrow.

Antibody-induced T cell depletion for suppression of graft-versus-host reactions is still little investigated in canine bone marrow transplantation because of certain logistic difficulties in the production of suitable monoclonal antibodies (mAbs).* This is also why, contrary to the immune system of mice and humans, canine cell populations involved in the immune regulation have not yet been well defined. Recently, several monoclonal antibodies have become available for characterization of dog lymphocytes. These antibodies allow the distinction between lymphoid and nonlymphoid cells: =DLy 6 (*1*); T and B lymphocytes = F3-20-7 (*2*); 1 WMD-1 (*3*); and a subset of T cells = DT2 (*4*), Aby 1A1 (*5*). Other mAbs-DLy1 (*1*) and Aby 6C6 (*6*) react with canine lymphoid and nonlymphoid cells.

This report describes 4 novel murine monoclonal antibodies that react with 2 distinct epitopes on an antigen of dog lymphocytes; this antigen is present on T lymphocytes but not on SIg^+ lymphocytes, nonlymphoid cells, or hemopoietic progenitors.

MATERIALS AND METHODS

Animals and cells. Lymphoid organs were obtained from 1–6-day-old outbred dogs. Thymus, lymph nodes, and femoral bone marrow were

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* Abbreviations used: ATG, antidog-thymocyte globulin; BM, bone marrow cell suspension; CFU_{GM}, granulocyte-macrophage progenitors; DTE, dithioerythritol; ELISA, enzyme-linked immunosorbent assay; GA, glutaraldehyde; GVHD, graft-versus-host disease; HRP, horseradish peroxidase; IgG, immunoglobulin; mAbs, monoclonal antibodies; MdT-mab, mouse-antidog T lymphocytes monoclonal antibody; MEM, minimum essential medium; MNS, mouse normal serum; NP-40, nonidet P-40; PBMC, peripheral blood mononuclear cells; PBS, Dulbecco's calcium- and magnesium-free phosphate-buffered saline; PLL, poly-L-lysine; SDS-PAGE, polyacrylamide gradient gel electrophoresis in sodium dodecyl sulphate; SPA-SRBC, staphylococcal protein-A–coated sheep red blood cells.

minced and passed through a stainless-steel mesh, and the cell suspension separated on Percoll 1.040 g/cm^3 in order to remove dead cells and cell debris. Contaminating erythrocytes from bone marrow were removed by separation on Percoll 1.085 g/cm^3. Peripheral blood was obtained from beagles bred in the kennels of the Gesellschaft für Strahlen- und Umweltforschung. Mononuclear cells were obtained following separation on Percoll 1.078 g/cm^3 by centrifugation at 1000 × g for 15 min.

Balb/c mice, originally obtained from Jackson Laboratories (Bar Harbor, ME), were raised and maintained in our breeding facilities.

Cell fusion and screening for antibodies. The method of Köhler and Milstein (*7*) was applied as modified by Fazekas (*8*) using NS-1 myeloma and spleen cells from Balb/c mice immunized with canine thymocytes. Primary screening was performed in a radioimmunoassay using dog thymocytes as target cells (*9, 10*).

Proof of Specificity of Mouse-Antidog T Lymphocytes Monoclonal Antibodies (MdT-mAbs) and Tissue Distribution of Antigen Recognized by MdT-mAbs

Slides. Multispot poly-L-lysine (PLL) coated slides, preparations of target monolayer and glutaraldehyde (GA) fixation were performed as described elsewhere (*11*).

Preparation of SIg$^+$ cells. An excess of PLL was rinsed and 20 μl of the IgG fraction of sheep-antidog IgG (Paesel, Frankfurt, FRG), adjusted to a concentration of 10 μg/ml in 0.05 M Tris buffer, pH 9.4, were pipetted onto each spot. After overnight incubation at 4°C, the spots were washed with Dulbecco's calcium- and magnesium-free phosphate-buffered saline (PBS), and the free binding sites of PLL blocked with undiluted FCS. The spots were washed again and 10 μl of cell suspension, at a concentration of 7–10×10^6 cells/ml of minimum essential medium (MEM), 5% fetal calf serum (FCS), added to each spot. After 1 hr sedimentation in a moist chamber, the slide was turned onto the reverse side in order to allow sedimentation of unbound cells into the medium for 20 min. The slides were gently dipped in PBS and fixed for 15 min with 0.05% GA in PBS.

Immunocytochemistry. The cells previously immobilized and fixed on PLL-coated slides were incubated with (1) mAb under test; (2) affinity-purified rat-antimouse-Ig horseradish peroxidase (HRP) (Jackson, Avondale, PA); (3) affinity-purified goat-antirat-Ig HRP (Jackson) antibodies. For staining the SIg$^+$ cells, the spots were incubated with rabbit-antidog (Miles, München, FRG) followed by goat-antirabbit-Ig HRP (Tago, Burlinghame, CA) antibodies.

The peroxidase reaction with 3-amino-9-ethylcarbazole, counterstaining, and sealing was performed as described elsewhere (*11*). In some experiments, the endogenous peroxidase was counterstained with 4 chloro-1-naphtol as described previously (*12*).

Immunohistochemistry. Tissue blocks of thymus, spleen, and lymph nodes taken from dogs aged 6–8 weeks were rapidly frozen in liquid nitrogen. Frozen sections were cut at 7 μm in a cryostat at −20°C, air dried, and fixed in acetone for 5 min. The sections were incubated with the following: (1) undiluted supernatants, or diluted NS 1 ascites for negative controls; (2) biotinylated sheep-antimouse-Ig (Amersham, Braunschweig, FRG); (3) peroxidase labeled avidin (Vector, Burlington, CA). B-cell regions were demonstrated by incubation with the following: (1) rabbit-antidog-Ig (Nordic, Dietzenbach, FRG); (2) goat-anti-rabbit-Ig in excess; (3) peroxidase-antiperoxidase-complex. The sections were incubated in each case for 60 min at room temperature. Peroxidase activity was demonstrated as described above under immunocytochemistry.

Microcytotoxicity assay. The microdroplet cytotoxicity test technique was performed as described in detail elsewhere (*13*). Rabbit complement absorbed with dog thymocytes and lymph node cells (250 mg cells/ml complement) was used.

Molecular weight estimation of antigen recognized by MdT monoclonal antibodies. SDS-polyacrylamide electrophoresis was carried out on discontinuous vertical slab gels (SDS-PAGE) as described by Laemmli (*14*). Linear gradient gels of 7–14% T, 2,67% C were prepared.

Dog thymocytes solubilized with nonidet P-40 (NP-40) as described previously (*15, 16*) were diluted in 4 times concentrated sample buffer (final concentration 50 mM Tris, 1% SDS, 20 mM dithioerythritol [DTE], 8% glycerol, 0.001% bromphenolblue pH 6.8, for nonreducing conditions DTE was omitted), denatured for 3 min at 100°C, and applied as equivalent of 4×10^6 cells/lane. After electrophoresis, the gel was equilibrated in 25 mM Tris, 192 mM glycine, 20% methanol buffer and blotted to NC membrane (Schleicher u. Schüll, Dassel, FRG) for 2 hr at 25 V/cm. After soaking in PBS, 1% nonfat dry milk to saturate nonspecific protein-binding sites, the sheet was cut into strips, each of which was incubated with the following: (1) mAb under test or irrelevant control mAb; (2) rat-antimouse Ig-HRP; (3) goat-antirat Ig-HRP. The peroxidase reaction with 4-chloro-1-naphtol was performed as described elsewhere (*12*).

Identification of binding determinants on the MdT antigen by competitive inhibition of binding. Solubilized membranes of canine thymocytes were immobilized on microtiter plates as described by Noteboom et al. (*17*). Nonspecific binding sites were blocked with 10% mouse normal serum (MNS) in PBS. The competitive inhibition assay was performed as described elsewhere (*18*) using biotinylated immunoglobulin (IgG) fraction of MdT monoclonal antibodies.

Hemopoietic progenitor cell activity (CFU_{GM}) in bone marrow cell suspension. MdT-P_1 or antidog thymocytes globulin (ATG) positive cells from canine bone marrow cell suspension (BM) were separated using the staphylococcal protein-A-coated sheep red blood cells (SPA-SRBC) technique (*19*) and density gradient centrifugation on Percoll 1.085 g/cm^3 by 1000 × g for 15 min. The cells from the interphase and pellet were assayed for CFU_{GM} activity as described by Pike et al. (*20*). The number of CFU_{GM} was estimated per 100,000 bone marrow cells in the original suspension and in each fraction after separation.

RESULTS

Isolation and immunochemical characterization of antibodies specific for canine lymphocytes. Out of 960 plated wells, 9 were T specific as estimated in immunohistology on thymus, lymph nodes and spleen sections, and immunocytochemistry on SIg$^+$ blood lymphocytes, cells of myelo- and erythropoietic lineage in the bone marrow and platelets. We describe 4 of these hybridomas, designated: MdT-P_1, MdT-P_2, MdT-Q_1, MdT-Q_2.

The subclass of the mAbs described was identified in double immunodiffusion using appropriate antimouse immunoglobulins as: MdT-P_1 IgG2b, MdT-P_2 IgG2a, MdT-Q_1 IgG2b, MdT-Q_2 IgG2b, all kappa.

These 4 MdT-mAbs recognized the same heterogeneous antigen of canine thymocytes. "Western blotting" of solubilized thymocytes have shown an antigen with band clusters at 90–120 kD and 140–160 kD. The MdT-P_1 mAb recognized additionally 2 bands of 33 and 66 kD molecular weight, presumably due to the high affinity of this antibody and in this way increased sensitivity of the system (Fig. 1). The same pattern has been observed under nonreducing conditions (not shown). As internal control, the F3-20-7 anticanine Thy-1 (*2*) (Serotec Ltd., Blackthorn Bicester, U.K.) was included. The molecular weight of canine Thy-1 antigen has previously been estimated as 24 kD (*2*); using our method, this antigen was estimated as 31 kD (Fig. 1).

Identification of topographically separate determinants on the MdT antigen by competitive inhibition of binding. Competitive inhibition ELISA assays using biotinylated MdT monoclonal antibodies were set up to determine whether the binding of one unlabeled antibody to the antigen interfered with the binding of another labeled antibody. Figure 2 shows that mAbs MdT-Q_1, -Q_2 and MdT-P_1, -P_2, on the other hand, defined 2 separate binding sites on the MdT antigen. MAbs MdT-Q_1 and MdT-

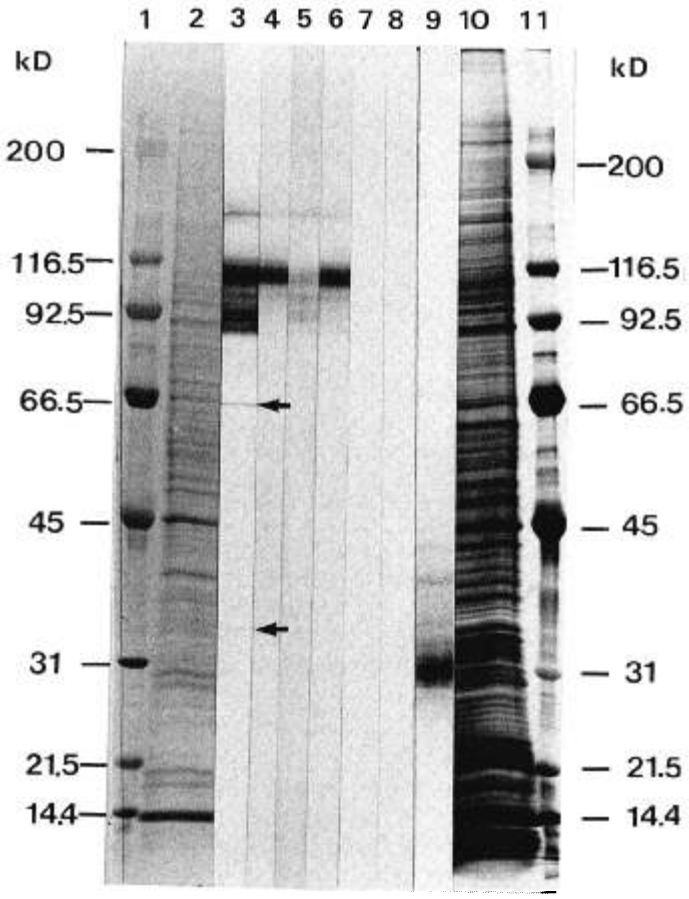

FIGURE 1. Immunoblotting of canine thymocytes surface membrane components. Lane 1–2: after SDS-PAGE fractionation, molecular weight protein standards and solubilized surface membrane components of canine thymocytes were blotted to NC-membrane and stained with Amidoblack 10B. Lane 1, molecular weight protein standards (Bio-Rad) lysosyme 14.4 kD-soybean trypsin inhibitor-21.5 kD, carbonic anhydrase-31 kD ovalbumin 45 kD, bovine serum albumin-66.2 kD, phosphorylase B-92.5 kD, β-galactosidase-116.25 kD, myosin-200 kD; lane 2, solubilized thymocytes membranes-equivalent of 4×10^6 cells. Lane 3–9: after SDS fractionation and electroblotting, solubilized thymocytes membranes—equivalent of 4×10^6 cells—were immunologically probed as described in the *Materials and Methods* section. Lane 3, MdT-P_1; lane 4, MdT-Q_2; lane 5, MdT-P_2; lane 6, MdT-Q_1; lane 7, irrelevant MRC-Ox7 mAb; lane 8, diluting medium; lane 9, F3-20-7 anticanine Thy-1. Lane 10–11: after SDS fractionation, molecular weight protein standards and solubilized surface membrane components were stained in combined silver-coomassie stain. Lane 10, solubilized thymocytes membranes-equivalent of 4×10^6 cells; lane 10, molecular weight protein standards as in lane 1. SDS-PAGE fractionation was performed under reducing conditions using DTE.

Q_2 were found to be mutually competitive: unlabeled MdT-Q_2 inhibited the binding of biotinylated MdT-Q_1 as completely as did unlabeled MdT-Q_1 itself. MdT-P_1 and MdT-P_2 antibodies, on the other hand, displayed only slight inhibition in the high concentration area (Fig. 2A). Figure 2B shows the inhibition of the binding of biotinylated MdT-Q_2 monoclonal antibody. The inhibition pattern corresponds very well to that in Figure 2A. Mutual competition in the case of MdT-P_1 and MdT-P_2 antibodies was markedly asymmetrical, MdT-P_1 being a better competitor of MdT-P_2 than MdT-P_2 itself (Fig. 2D) and MdT-P_2 being a weak competitive inhibitor of MdT-P_1 (Fig. 2C). The mAbs MdT-Q_1 and MdT-Q_2 show only slight inhibition at the highest concentrations. The irrelevant MRC-Ox7 mAb (*21*) has no inhibitive effect whatsoever.

Distribution of MdT Antigen on Canine Lymphoid Tissue

Single cell suspensions. Table 1 depicts the reactivity pattern of MdT-mAbs with peripheral blood mononuclear cells (PBMC), thymocytes, lymph node cells, and bone marrow cells. Analysis of enriched SIg^+ blood lymphocytes showed that none of the MdT-mAbs recognized B lymphocytes. The enrichment of SIg^+ blood lymphocytes on the multispot slides was increased to 98%. In addition, the crossreactivity of MdT-mAbs with blood monocytes, granulocytes, erythrocytes, platelets, as well as cells of myelo- and erythropoietic lineage in the bone mar-

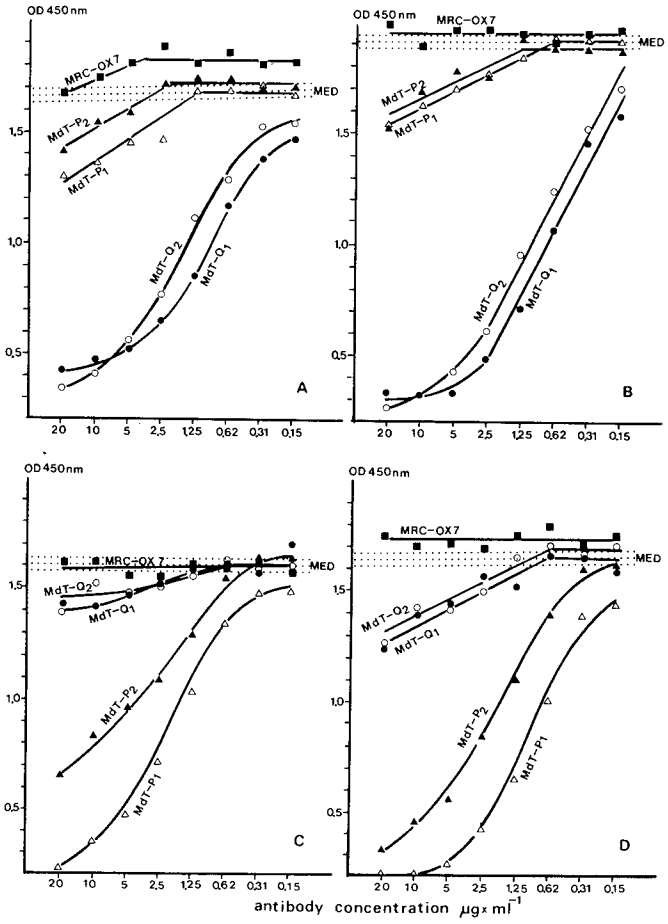

FIGURE 2. Competitive inhibition of binding of biotin labeled MdT mAbs to solubilized membranes of dog thymocytes. Immobilized canine thymocytes NP-40 extract was incubated with serial twofold dilutions of purified unlabeled competitor antibodies in concentrations ranging from 20 to 0.015 µg/ml. Purified biotinylated antibody was added in constant concentration to the unlabeled competitor antibodies. The biotinylated antibody had the following specificity: MdT-Q_1 in A, MdT-Q_2 in B, MdT-P_1 in C, MdT-P_2 in D. The inhibition of the binding was estimated using avidin HRP; 100% binding (uninhibited control) was achieved by including irrelevant MRC-OX7 antibody, or diluting medium in the first incubation step. For the positive, inhibited control unlabeled mAb was used in the first incubation step and the same, biotinylated, in the second one. ●——● MdT-Q_1; ○——○ MdT-Q_2; △——△ MdT-P_1, ▲——▲ MdT-P_2; ■——■ MRC-Ox7, medium (dotted lines).

TABLE 1. Reactivity of MdT monoclonal antibodies in indirect peroxidase assay

Antibody[a]	MdT-P_1	MdT-P_2	MdT-Q_1	MdT-Q_2	Rabbit-antidog IgG[b]
Blood monocytes, granulocytes, erythrocytes, platelets	0 (9)	0 (8)	0 (9)	0 (8)	ND
Blood lymphocytes	58±4.9 (13) /55–69/	48±6.3 (7) /40–57/	43±7.3 (10) /35–57/	38±5.0 (9) /30–45/	24±6.3 (9) /16–34/
SIg^+ blood lymphocytes	2 (3)	0 (3)	0 (3)	0 (3)	98±1.0 (3)
Thymocytes	91±3.4 (4) /88–95/	81±3.2 (4) /76–83/	74±6.0 (3) /68–80/	67±9.0 (3) /58–76/	3.5±2.3 (3) /1–6/
Lymph node lymphocytes	72±6.6 (3) /64–76/	56±1.1 (3) /55–57/	49±6.1 (3) /42–53/	40±8.9 (3) /34–50/	21±3.0 (3) /18–24/
Bone marrow lymphocytes	2 (3)	0 (3)	0 (3)	0 (3)	26±3, 5 (3)
Bone marrow cells (myelo- and erythropoietic lineage)	0 (3)	0 (3)	0 (3)	0 (3)	ND

[a] Percentage-Mean ± SD (No. different dogs)/range/-of cells labeled after incubation with MdT ascitic fluids followed by second rat-antimouse IgG HRP and third goat-antirat IgG HRP antibodies.

[b] Percentage of cells labeled after incubation with rabbit-antidog IgG followed by goat-antirabbit IgG HRP (ND, not done).

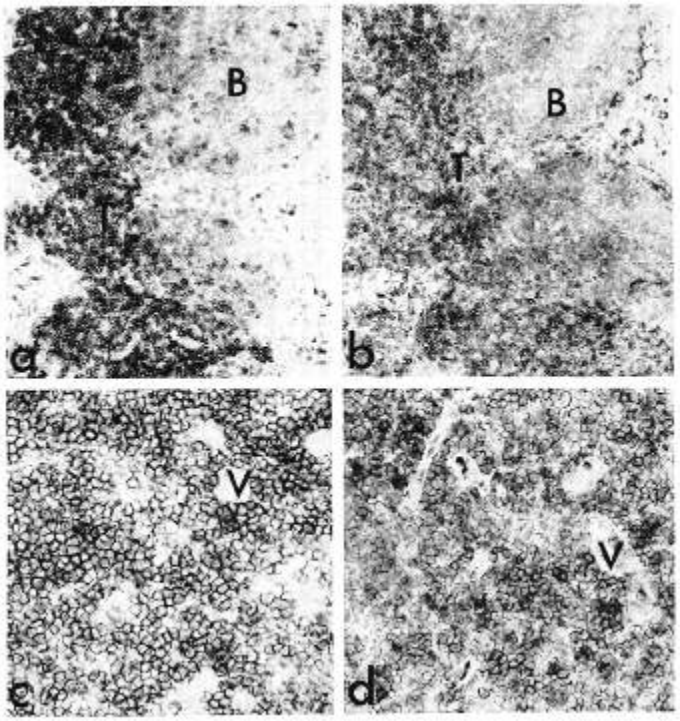

FIGURE 3. Lymph node sections stained with antibody MdT-P_1 (a, c) and MdT-P_2 (b, d). B cell cortical zones (B) are almost free of labeled cells, paracortical T cell zones (T) contain a majority of MdT-P_1 labeled lymphocytes (a, c) and a lower percentage of lymphocytes labeled with MdT-P_2 (b, d). V: high endothelial venules = typical vessel of T cell areas a, b ×70, c, d ×175.

row, was tested by way of endogenous peroxidase counterstaining with 4-Cl-1-naphtol. Table 1 shows that none of the MdT-mAbs react with nonlymphoid cells. The complement-dependent cytotoxicity tested against PBMC, thymocytes, and lymph node cells was restricted to thymocytes; all the MdT-mAbs lysed over 90% of thymocytes but not peripheral lymphocytes (data not shown).

Frozen sections. Labeling patterns of the 4 monoclonal antibodies differed in thymic medulla and in peripheral lymphoid organs. The vast majority of thymic cortex was labeled strongly by antibodies MdT-Q_1, MdT-P_2, MdT-P_1, and more weakly by MdT-Q_2. Antibody MdT-P_1 also strongly labeled the majority of medullary thymocytes and of paracortical T cell areas in lymph nodes (Fig. 3) and in the periarteriolar zone in spleen white pulp. Antibody MdT-P_2 distinctly stained about 60% of thymic medulla cells and lymph node (Fig. 3) as well as spleen T cell zones, leaving the remaining cells unstained. Antibody MdT-Q_1 distinctly labeled about 50% of thymic medulla, whereas the remaining half was stained very weakly.

Peripheral T cell areas in lymph node and spleen showed a similar staining as in the thymic medulla. Antibody MdT-Q_2 stained—to differing extents—only 30–40% of thymic medulla, lymph node paracortex, and the periarteriolar zone in spleen white pulp.

Studies of antibody activity on canine hemopoietic progenitors. In order to study the expression of MdT antigen on hemopoietic progenitor cells, a CFU_{GM} assay was set up. Figure 4 shows that subsequent to SpA rosetting and density gradient separation of BM cells incubated with saturating concentrations of MdT-P_1, nearly all CFU_{GM} were found in the interphase. Only 3% of CFU_{GM} were found in the pellet (containing MdT-P_1 positive cells). Contrary to this, CFU_{GM} activity in bone marrow cells separated after incubation with ATG was found mainly in the pellet (95%); the interphase contained only about 3% of CFU_{GM}. The number of CFU_{GM} per 100,000 unseparated BM cells was taken to represent 100% CFU_{GM} activity.

DISCUSSION

In the present study, we describe 4 monoclonal antibodies, recognizing the same heterogeneous antigen on canine T cells. The same patterns under reducing and nonreducing conditions have shown that the differences in molecular weight of the revealed bands could not be explained through disulphide interactions. Further investigations are required, if the described heterogeneity is due to differences in the degree of glycosylation and/or hydrophobic interactions.

The epitope analysis of MdT antigen shows that there are 2 distinct binding regions (P, Q). Complete mutual competition of MdT-Q_1 and MdT-Q_2 pair indicates that MdT-Q_1 and MdT-Q_2 recognize the same or very closely linked epitope(s). This was also the case with the MdT-P_1, -P_2 pair. The mutual competition within this binding group was, however, asymmetrical—with MdT-P_1 being a better competitor of MdT-P_2 than

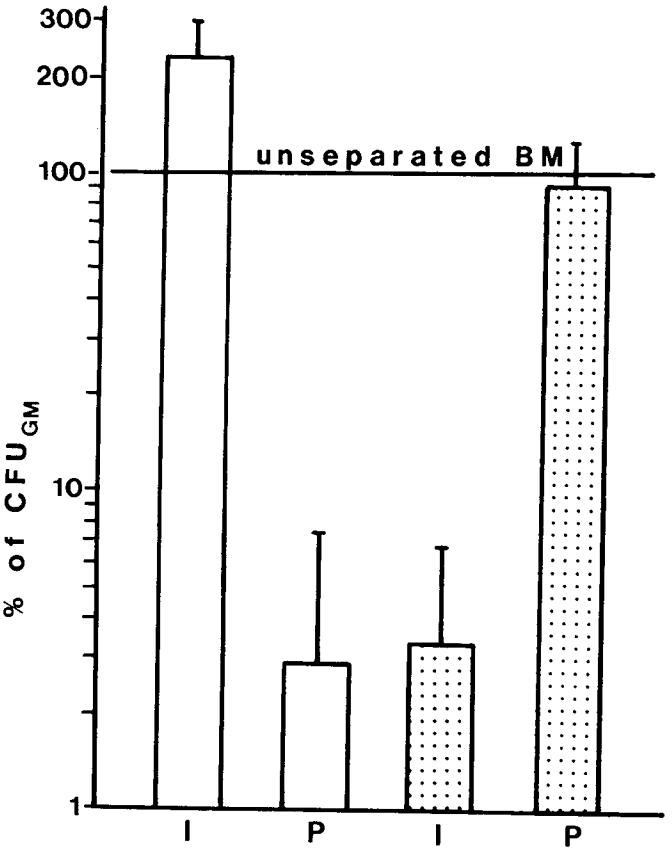

FIGURE 4. CFU_{GM} activity in bone marrow cell suspension before and after depletion of MdT-P_1 or ATG positive cells. Percentage (mean of 5 experiments +SD) of CFU_{GM} in bone marrow cell suspension after indirect SpA rosetting and density gradient separation of □ MdT-P_1 positive or ATG positive (dotted columns) cells. I: interphase, P: pellet. The number of CFU_{GM}/100,000 unseparated bone marrow cells was taken to represent 100% CFU_{GM} activity.

MdT-P_2 itself. Time kinetics in the case of MdT-P_1, -P_2 pair show that MdT-P_2 was displaced by MdT-P_1 to an extent proportional to the incubation time (data not shown). This could indicate low avidity of MdT-P_2.

To determine the distribution of MdT antigen on single cell suspensions, we used the multispot immunoperoxidase assay that combines surface labeling with excellent morphology, so that the exact cell lineage can be quickly determined. The problem of endogenous peroxidase, which complicates evaluations of bone marrow and to some extent of PBMC, was satisfactorily overcome by counterstaining with 4-Chloro-1-naphtol. Counterstaining rather than inhibiting endogenous peroxidase also allows classification of certain cells that are difficult to discriminate otherwise.

The reactivity of our MdT antibodies in immunocytochemistry included peripheral blood T lymphocytes, thymocytes, lymph nodes, and bone marrow lymphocytes. MdT-mAbs labeled neither SIg positive lymphocytes nor monocytes, platelets or cells of myelo- and erythropoietic lineage in the bone marrow from normal dogs (Table 1). The T cell specificity of these reagents was also supported by immunohistochemistry performed on thymus, spleen, and lymph node sections, where the reactivity of the MdT-mAbs was restricted to T cell areas only. It is interesting to note that mAbs directed against the same epitope exhibited a different binding pattern when tested on single cell suspension of lymphoid organs, presumably due to different avidity of these monospecific reagents.

As a single monoclonal IgG, our MdT-mAbs were generally able to fix complement in the complement-dependent cytotoxicity test; approximately 90% of the thymocytes were killed by all 4 antibodies, independent of their isotype. These antibodies also bound human Clq, a subunit of the first component of the classic pathway, using canine thymocytes as target. In contrast, none of our mAbs lysed peripheral lymphocytes, which is probably due to a lower antigen density on peripheral lymphocytes.

Transplantation of marrow from DLA homozygous dogs to 1-DLA-haplotype mismatched littermates was observed by us with absence of graft-versus-host disease (GVHD) in more than half of the dogs when bone marrow was incubated with absorbed rabbit ATG in the absence of complement (*22, 23*). However, numerous monoclonal anti-T lymphocyte antibodies showed little suppression of GVHD in mice (*24, 25*) and humans (*26*), following in vitro treatment in the absence of complement. A high affinity of monoclonal reagents for Clq seems to be an important factor for efficacy in the clearance of antibody-coated T cells in the marrow recipient (*27*). Recently, we have shown (*28*) that in vivo injection of monoclonal antibodies with high affinity for Clq before transfusion of the marrow cells suppresses GVH and host-versus-graft reactions in 2-haplotype H-2, IA mismatched mice. Hughes-Jones and colleagues (*29*) demonstrated that pairs of synergistic antibodies are able to increase the binding constant between Clq and mAbs bound at the cell surface, and to increase the rate of Cl activation. Defining canine T cell specific antigens with epitopes for synergistic pairs of antibodies may prove useful for in vitro and in vivo T cell depletion in bone marrow transplantation. Our studies of antibody activity on canine hemopoietic progenitor cells in CFU_{GM} using MdT-P_1 and a SPA-SRBC rosetting technique showed no reduction of CFU_{GM} in T-cell–depleted bone marrow.

Additionally, a lethally irradiated dog receiving a bone marrow autograft depleted of MdT-P_1, positive cells showed engraftment and complete recovery of marrow function (unpublished observation). This indicates that MdT-P_1 antibody spares stem cells and may prove of value for the study of treating GVHD in a preclinical animal model.

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