Ann Hematol (1991) 63: 223–226

Annals of
Hematology

Short Communication

Immunological characterization of canine hematopoietic progenitor cells*

J. Hahn[1], H. J. Kolb[1,4], M. Schumm[1], K. Beisser[3], J. Ellwart[2], P. Rieber[5], J. Maldacker[1], N. Schwella[1], L. K. Lösslein[3], E. Holler[3,4], W. Wilmanns[3,4], and S. Thierfelder[1]

[1] Institut für Immunologie; [2] Experimentelle Hämatologie; [3] Klinische Hämatologie, Hämatologikum der GSF, Marchioninistrasse 25, W-8000 München 70, FRG
[4] Medizinische Klinik III, Klinikum Großhadern, Marchioninistrasse 15, W-8000 München 70, FRG
[5] Institut für Immunologie, Ludwig-Maximilian-Universität München, Goethestrasse 31, W-8000 München 2, FRG

Received and accepted August 23, 1991

Summary. Canine hematopoietic progenitor cells were characterized by separation with monoclonal antibodies. Depleted and enriched fractions were studied for growth of CFU-GM in semisolid agar and for repopulating capacity of lethally irradiated dogs. CFU growth was not reduced by depletion of marrow using monoclonal antibodies F 3-20-7 (anti-dog Thy-1), MT606 (anti-human CD6), and IOT2a (anti-human DR). CFU growth was variable following treatment with the anti-canine T-cell antibody MdT-P1 and immunomagnetic bead separation. It was regularly enriched when MdT-P1 treatment was followed by immunorosetting with staphylococcal protein A-loaded sheep red blood cells and density gradient separation. Lethally irradiated dogs were reconstituted by autologous marrow depleted of MdT-P1-positive cells using immunorosetting and density gradient centrifugation, whereas immunomagnetic bead-depleted marrow was ineffective. Fluorescence-activated cell sorting showed enrichment of hematopoietic progenitor cells in the weakly MdT-P1-positive fraction.

Key words: Hematopoietic progenitor cells – Dog – Monoclonal antibodies – CFU-GM – Autologous bone marrow transplantation

Introduction

Studies on the physiology of human and mouse hematopoiesis have been facilitated by the development of monoclonal antibodies and methods of positive selection [7, 8, 35]. Hematopoietic progenitor cells of the dog are less well characterized, despite the wide use of dogs in preclinical transplantation studies. Some human DR antibodies cross-reactive with canine cells did not cross-react with hematopoietic progenitor cells [26]; others did and could be used for positive selection [4, 33]. Some antibodies against canine T cells suppressed the growth of hematopoietic precursor cells [26]; others did not suppress colony growth [25].

We report here our studies with two canine and two cross-reactive human antibodies and separation methods for negative and positive selection of canine hematopoietic progenitor cells.

Materials and methods

Monoclonal antibodies

MdT-P1, a murine antibody of the IgG2b isotype, recognizes up to 95% of canine thymocytes and 70% of canine blood lymphocytes. Its specificity for canine T lymphocytes has been demonstrated by immunohistology of lymph nodes and functionally in mixed lymphocyte culture (MLC) and regulation of B-cell function in the hemolytic plaque assay [17, 23, 25].

MT606, an IgG2b molecule, directed against human CD6 [18], an antigen expressed by the majority of human T-lymphocytes and a small subpopulation of B lymphocytes, cross-reacts with up to 60% of canine peripheral blood lymphocytes.

F3-20-7, a murine antibody of the IgG1 isotype, was described by McKenzie and Fabre [22] as specific for canine Thy-1-antigen.

IOT2a (Dianova, Hamburg), an IgG2b antibody directed against HLA-DR, cross-reacts with approximately 80% of canine lymphocytes. Several other HLA-DR antibodies cross-reacting with canine MHC class II have been described [9–11].

Separation of antibody-coated cells

Immunorosetting and density gradient separation. An indirect rosetting technique was performed using staphylococcal-protein A-coated sheep red blood cells (SRBC) to label MdT-P1-positive cells. Rosette-forming cells (RFC) were layered over Percoll (d = 1.085; Percoll, Pharmacia LKB GmbH, Freiburg) and separated by centrifugation with 1000 g for 20 min [23].

Address for correspondence: H.-J. Kolb, Medizinische Klinik III, Klinikum Großhadern, Marchioninistrasse 15, W-8000 München 70, FRG

* Nonreviewed contribution to the annual meeting of the German and Austrian Societies of Hematology.

This work was supported by the Wilhelm Sander Foundation, AZ 84.020.1

This work is part of a doctoral thesis by J. Hahn, Ludwig-Maximilian-University Munich

Immunomagnetic beads. Immunomagnetic beads coupled with goat-anti-mouse globulin (Dynabeads M-450, Dianova, Hamburg) were used to remove antibody-labeled cells in a magnetic field [13, 14, 37].

Cell sorter. Bone marrow cells were incubated with MdT-P 1. Following two washes, they were incubated with F(ab')2 fragments of goat-anti-mouse IgG conjugated to fluorescein isothiocyanate (GaM-FITC, Sigma Chemie, Deisenhofen). To select MdT-P 1 low and high positive cells a fluorescence-activated cell sorter (FACS-Star Plus, Becton-Dickinson, Heidelberg) was used.

CFU-GM assay. Cultures of hematopoietic progenitor cells were done in an agar-culture system adapted for the dog [19], using conditioned medium of PHA-stimulated lymphocytes (PHA-LCM) or sera of granulocytopenic dogs (CSA) for stimulation.

Bone-marrow transplantation. Autologous transplantation of depleted bone marrow was performed in healthy beagle dogs raised in the kennels of the *Gesellschaft für Strahlen- und Umweltforschung*, Neuherberg. They were regularly dewormed and vaccinated against distemper, canine hepatitis, leptospirosis, and parvovirus. Bone marrow was given 12–24 h after total body irradiation with 10 Gy. This dose is lethal if marrow is not given subsequently.

Results

Removal of cells positive for F3-20-7 (canine Thy-1) or IOT2a (human class-II-antibody) using the immunomagnetic bead technique resulted neither in enrichment nor in depletion of CFU-GM (Table 1). In contrast, depletion with the human CD 6 antibody MT606 resulted in an enrichment of CFU-GM. Results with the canine T-cell antibody MdT-P 1 were inconsistent. In some experiments CFU-GM were enriched, in others depleted or unchanged. These results are in contrast to our previous experience with immunorosetting and density gradient centrifugation. In those experiments enrichment of hematopoietic progenitor cells in the MdT-P 1-negative fraction was consistently found [23, 25]. The depletion with immunomagnetic beads may have more effectively removed T cells with their stimulatory activity on CFU-GM growth; alternatively, hematopoietic progenitor cells may express the MdT-P 1 antigen.

The addition of irradiated (40 Gy) peripheral blood lymphocytes (PBL) stimulated the CFU-GM growth of unseparated marrow as well as that of marrow depleted of F3-20-7-, MT606- or IOT2a-positive cells by immunomagnetic bead separation. In contrast, CFU-GM growth of MdT-P 1-depleted marrow was not stimulated by the addition of PBL (Table 2).

Subsequently, we sorted canine marrow cells into a fraction of cells weakly positive for MdT-P 1 and a fraction strongly positive for MdT-P 1 using a fluorescence-activated cell sorter. CFU-GM growth was absent in the strongly positive fraction but enriched in the weakly positive fraction (Table 3).

Results of autologous bone marrow transplantation using antibody-depleted marrow were in agreement with the in vitro results (Table 4). Bone marrow depleted of MdT-P 1-positive cells by immunorosetting and density gradient centrifugation contained many hematopoietic progenitor cells and led to hematological reconstitution of two lethally irradiated dogs. Bone marrow depleted by immunomagnetic beads failed to reconstitute a dog. This dog died on day 17 of marrow aplasia. As a control, MT606-depleted marrow led to an uneventful recovery of hematopoiesis.

Discussion

Several human DR antibodies cross-react with canine lymphocytes [9–12, 20] and two canine Ia antibodies have been described [1] as cross-reacting with human DR molecules, thus confirming the close relationship of the MHC class-II molecules of both species. In contrast to human and murine T lymphocytes, canine T lymphocytes appear to express class-II antigens constitutively [9–12, 20]. However, different human DR antibodies are reported, one cross-reacting with canine T and B lymphocytes and another cross-reacting with B cells and only activated T lymphocytes [11]. These different patterns may explain the variable results with hematopoietic progenitor cells in canine bone marrow [4, 26, 33]. Moreover, the considerable allelic variation in the DLA-D region [29–31] may also explain the different results with various human DR antibodies. Antibody IOT2a used in this study does react with more than 80% of unstimulated, more than 90% of stimulated peripheral blood lymphocytes, and about 20% of bone marrow cells, but not with CFU-GM. The anti-

Table 1. CFU-GM enrichment in antibody-depleted bone marrow using immunomagnetic-beads

Antibody for depletion	n =	CFU-GM[a] before depletion	Enrichment[b] after depletion	
F3-20-7 (= canine Thy-1)	3	158 ± 53[c]	1.0 ± 0.44[d]	(0.53–1.41)[e]
IOT2a (= HLA-DR)	8	88 ± 29	1.05 ± 0.64	(0.51–2.22)
MT606 (= human CD6)	7	138 ± 167	1.61 ± 0.61	(0.68–2.29)
MdT-P1 (= canine T cells)[f]	6	47 ± 41	0.13 ± 0.1	(0 –0.25)
	3	76 ± 49	0.9 ± 0.46	(0.53–1.42)
	5	70 ± 59	7.05 ± 6.6	(2.48–18.5)

[a] CFU-GM growth/50000 mononuclear cells
[b] Enrichment = CFU-GM after depletion / CFU-GM before depletion
[c] Mean ± standard deviation of CFU-GM growth in n experiments
[d] Mean ± standard deviation of enrichment in n experiments
[e] Range of enrichment in n experiments
[f] Grouped ranges of enrichment: < 0.5 0.5–2, > 2

gen recognized by this antibody is expressed on canine T lymphocytes but not on hematopoietic progenitor cells. Cross-reactions of human CD 6 antibodies with canine T lymphocytes have not yet been described. Depletion of CD 6-positive cells from the marrow seems to be promising for prevention of graft-versus-host disease. Stable mixed chimerism has been described in man following transplantation of CD 6-depleted marrow [5,27,28,34]. Our CD 6 antibody does not cross-react with canine hematopoietic progenitor cells, and may be useful for preclinical studies of T-cell depletion.

The canine T-cell antibody MdT-P 1 gave inconsistent results using immunomagnetic beads for depletion. This was not due to a more effective depletion of accessory cells necessary for CFU-GM growth, since the addition of PBL was not effective (Table 2). Obviously immunomagnetic beads removed also MdT-P 1-weakly-positive cells. These cells, however, did not form SRBC-rosettes stable enough to be separated by density-gradient centrifugation.

Table 2. Enhancement of CFU-GM growth using autologous, irradiated (40 Gy) PBL before and after immunomagnetic beads and antibody depletion

Antibody for depletion	n =	Enhancement[a]	
		Before depletion	After depletion
F 3-20-7	3	1.47 ± 0.21	1.77 ± 0.45
IOT 2a	8	1.48 ± 0.24	2.01 ± 0.49
MT 606	3	1.32 ± 0.34	1.66 ± 0.79
MdT-P 1	8	1.37 ± 0.31	1.08 ± 0.60

[a] Enhancement = CFU-GM stimulated with LCM + PBL/CFU-GM stimulated with LCM
Mean ± standard deviation of n experiments

Table 3. Distribution of CFU-GM growth in canine bone marrow before and after sorting of MdT-P 1-low- and -high-positive cells

Unsorted	MdT-P 1-low positive	MdT-P 1-high positive
12[a]	362	0

[a] CFU-GM/50 000 mononuclear cells

This finding of a T-cell antigen on canine hematopoietic progenitor cells corresponds well with the findings in other species, mice and rats [3,6,15,24,32,36]. The expression of T-cell markers on human hematopoietic precursor cells is still a matter of discussion [2,16,21].

We have shown that canine hematopoietic progenitor cells can be enriched in the MdT-P 1-weakly-positive fraction of bone marrow cells. This allows further characterization of canine hematopoietic progenitor cells in culture and in preclinical transplantation experiments.

Acknowledgements. The authors thank Mrs. I. Stenzel, A. Arco v. Zinneberg, and L. Gatsch for their excellent technical assistance.

References

1. Alejandro R, Shienvold FL, Latif Z, Esquenazi V, Miller J, Mintz D (1984) Monoclonal antibodies recognizing canine and human Ia-like antigens. Transplantation 38: 542–544
2. Andrews RG, Singer JW, Bernstein ID (1990) Human hematopoietic precursors in long-term culture: single CD 34+ cells that lack detectable T cell, B cell, and myeloid cell antigens produce multiple colony-forming cells when cultured with marrow stromal cells. J Exp Med 172: 355–358
3. Basch RS, Berman JW (1982) Thy-1 determinants are present on many murine hematopoietic cells other than T cells. Eur J Immunol 12: 359–364
4. Berenson RJ, Bensinger WI, Kalamasz D, Schuening F, Deeg HJ, Graham T, Storb R (1987) Engraftment of dogs with Ia-positive marrow cells isolated by avidin-biotin immunoadsorption. Blood 69: 1363–1367
5. Bosserman LD, Murray C, Takvorian T, et al. (1989) Mechanism of graft failure in HLA-matched and HLA-mismatched bone marrow transplant recipients. Bone Marrow Transplant 4: 239–245
6. Boswell HS, Wade PM Jr, Quesenberry PJ (1984) Thy-1 antigen expression by murine high-proliferative capacity hematopoietic progenitor cells. I. Relation between sensitivity to depletion by Thy-1 antibody and stem cell generation potential. J Immunol 8: 2940–2949
7. Civin CI, Strauss LC, Brovall Ch, Fackler MJ, Schwartz JF, Shaper JH (1984) Antigenic analysis of hematopoiesis. III. A hematopoietic progenitor cell surface antigen defined by a monoclonal antibody raised against KG-1a cells. J Immunol 133: 157–165
8. Civin CI, Banguenigo ML, Strauss LS, Loken MR (1987) Antigenic analysis of hematopoiesis. VI. Flow cytometric characterization of My 10-positive progenitor cells in normal human bone marrow. Exp Hematol 15: 10–17
9. Deeg HJ, Wulff JC, DeRose S, Sale GE, Braun M, Brown MA, Springmeyer SC, Martin PJ, Storb R (1982) Unusual distribu-

Table 4. Autologous transplantation of MdT-P 1- or MT 606-depleted bone marrow

Antibody	Depletion technique	Dog	Log depletion	MNC/kg ($\times 10^7$)	CFU/kg	Clinical outcome
MdT-P 1	SRBC[a]	N 875	> 2.43	3.39	n.e.	Complete recovery
	SRBC	N 865	2.8	1.3	57590	Complete recovery
	Beads[b]	120 N	> 2.83	6	3900	Died of aplasia on day 17 after transplantation
MT 606	Beads	57 N	> 2.2	3.48	32700	Complete recovery

[a] Immunorosetting using staphylococcal-protein A-coated sheep red blood cells (= SRBC) and density gradient centrifugation (d = 1.085).
[b] Immunomagnetic beads coated with goat-anti-mouse-antibodies were used to remove MdT-P 1- or MT 606-labeled cells in a magnetic field.

tion of Ia-like antigens on canine lymphocytes. Immunogenetics 16: 445–457
10. Doveren RFC, Buurman WA, Schutte B, Groenewegen G, Van der Linden CJ (1985) Class-II antigens on canine T lymphocytes. Tissue Antigens 25: 255–265
11. Doveren RFC, Van der Linden CJ, Spronken EEM, Groenewegen G, Buurman WA (1986) Canine MHC-class-II antigens on B and T lymphocytes. Tissue Antigens 27: 87–98
12. Doxiadis I, Krumbacher K, Neefjes JJ, Ploegh HL, Grosse-Wilde H (1989) Biochemical evidence that the DLA-B locus codes for a class-II determinant expressed on all canine peripheral blood lymphocytes. Exp Clin Immunogenet 6: 219–224
13. Egeland T, Albrechtsen D, Martin PJ, Hansen JA (1990) Immunomagnetic depletion of CD6+ cells from bone marrow and peripheral blood. Bone Marrow Transplant 5: 193–198
14. Gee AP, Lee C, Sleasman JW, Madden M, Ugelstadt J, Barrett DJ (1987) T lymphocyte depletion of human peripheral blood and bone marrow using monoclonal antibodies and magnetic microspheres. Bone Marrow Transplant 2: 155–163
15. Goldschneider I, Gordon LK, Morris RJ (1978) Demonstration of Thy-1 antigen on pluripotent hemopoietic stem cells in the rat. J Exp Med 148: 1351–1366
16. Haynes BF, Denning SM, Singer KH, Kurtzberg J (1989) Ontogeny of T-cell precursors: a model for the initial stages of human T-cell development. Immunol Today 10: 87–91
17. Hötzl C, Kolb HJ, Holler E, Hahn J, Beisser K, Mysliwietz J, Rieber P, Mempel W, Wilmanns W, Thierfelder S (1991) Functional characterization of canine lymphocyte subsets. Ann Hematol 63: 49–53
18. Knapp W, et al. (eds) (1990) Leukocyte typing, vol IV. Oxford University Press, Oxford
19. Konwalinka G, Peschel C, Geissler D, Boyd J, Tomaschek B, Odavic R, Braunsteiner H (1983) Myelopoiesis of human bone marrow cells in a micro-agar culture system: comparison of two sources of colony-stimulating activity (CSA). Int J Cell Cloning 1: 401–411
20. Krumbacher K, Van der Feltz MJM, Happel M, Gerlach C, Lösslein LK, Grosse-Wilde H (1986) Revised classification of the DLA loci by serological studies. Tissue Antigens 27: 262–268
21. Lansdorp PM, Sutherland HJ, Eaves CJ (1990) Selective expression of CD45 isoforms on functional subpopulations of CD34+ hemopoietic cells from human bone marrow. J Exp Med 172: 363–366
22. McKenzie JL, Fabre JW (1981) Studies with a monoclonal antibody on the distribution of Thy-1 in the lymphoid and extracellular connective tissues of the dog. Transplantation 31: 275–282
23. Maldacker J (1987) Charakterisierung und Rosettenseparation von T-Lymphocyten mit polyklonalem Antithymozytenglobulin (ATG) und monoklonalem Pan-T-Antikörper im Knochenmark zur Prophylaxe der Graft-versus-Host-Krankheit beim Hund. Dissertation, University of Munich
24. Miller BA, Lipton JM, Linch DC, Burakoff SJ, Nathan DG (1985) Thy-1 is a differentiation antigen that characterizes immature murine erythroid and myeloid hematopoietic progenitors. J Cell Physiol 123: 25–32
25. Mysliwietz JJ, Hoffmann-Fezer G, Thierfelder S, Kolb HJ, Maldacker J (1988) Recognition of two epitopes of an antigen present on canine T cells but not on hemopoietic progenitors by four monoclonal antibodies. Transplantation 45: 443–448
26. Prendergast MM, Bradstock KF, Broomhead AF, Hughes WG, Karbal A, Berndt MC, Tiver K (1986) Monoclonal antibody analysis of canine hemopoietic cells. Transplantation 41: 565–571
27. Ritz J, Takvorian T, Nadler LM, et al. (1988) Prevention of graft-versus-host-disease by selective T-cell depletion of bone marrow with anti-T12 monoclonal antibody. Blood 72 [Suppl 1]: 403 (abstract)
28. Roy DC, Tantravahi R, Murray C, Dear K, Gorgone B, Anderson KC, Freedman AS, Nadler LM, Ritz J (1990) Natural history of mixed chimerism after bone marrow transplantation with CD6-depleted allogeneic marrow: a stable equilibrium. Blood 75: 296–304
29. Sarmiento UM, Storb R (1988) Characterization of class-II alpha genes and DLA-D region allelic associations in the dog. Tissue Antigens 32: 224–234
30. Sarmiento UM, Storb R (1988) Restriction fragment length polymorphism of the major histocompatibility complex of the dog. Immunogenetics 28: 117–124
31. Sarmiento UM, Sarmiento JI, Storb R (1990) Allelic variation in the DR subregion of the canine major histocompatibility complex. Immunogenetics 32: 13–19
32. Schrader JW, Battye F, Scollay R (1982) Expression of Thy-1 antigen is not limited to T cells in cultures of mouse hemopoietic cells. Proc Natl Acad Sci USA 79: 4161–4165
33. Schuening F, Storb R, Goehle S, Meyer J, Graham T, Deeg HJ, Pesando J (1987) Canine pluripotent hematopoietic stem cells and CFU-GM express Ia-like antigens as recognized by two different class-II-specific monoclonal antibodies. Blood 69: 165–172
34. Soiffer RJ, Bosserman L, Murray C, Cochran K, Daley J, Ritz J (1990) Reconstitution of T-cell function after CD6-depleted allogeneic bone marrow transplantation. Blood 75: 2076–2084
35. Spangrude GJ, Heimfeld S, Weissman IL (1988) Purification and characterization of mouse hematopoietic stem cells. Science 241: 58–62
36. Thierfelder S (1977) Haemopoietic stem cells of rats but not of mice express Th-1.1 alloantigen. Nature 269: 691–693
37. Vartdal F, Kvalheim G, Lea TE, Bosnes V, Gaudernack G, Ugelstad J, Albrechtsen D (1987) Depletion of T lymphocytes from human bone marrow. Use of magnetic monosized polymer microspheres coated with T-lymphocyte-specific monoclonal antibodies. Transplantation 43: 366–371