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MONOCLONAL ANTIBODIES AS TOOLS FOR THE STUDY OF MONONUCLEAR PHAGOCYTES

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The myeloma X immune spleen cell hybrid technique of Köhle and Milstein (1, reviewed in 2) has given great impetus to the analysis of complex biological systems. For example, macrophages of one species such as the mouse may be injected into another species such as the rat. The resultant multispecific response to a large array of different macrophage surface molecules may then be resolved by cloning into a set of hybrid lines, each secreting a monoclonal antibody (MAb) recognizing a single antigenic determinant on a single cell surface molecule. Recently, a substantial number of antimacrophage MAb have been obtained that are already proving to be invaluable reagents of extraordinary specificity for the study of macrophage differentiation, function, and surface antigen structure

The properties of monoclonal antibodies defining murine an human macrophage differentiation antigens are summarized in Tables I and II, respectively. Most antibodies have been char acterized for expression on different leukocytes and cell line but not on nonhematopoietic tissues or on mononuclear phagocyt other than macrophages. None of these monoclonals binds to ly

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TABLE I. Rat Monoclonal Antibodies Defining Murine Monocytic Differentiation Antigens

Monoclonal antibody	Antigen designation	Antibody Chains	Antibody Subclass	Lysis	Antigen Polypeptide chains	Tissue distribution: Positive	Tissue distribution: Negative	Reference
M1/70	Mac-1	HL[a]	IgG_{2b}	weak	105,000 190,000	Exudate and resident peritoneal macrophages, 50% bone marrow cells, $P388D_1$, J774	Thymocytes, lymph node cells, 90-95% spleen cells, P815, B and T lymphoid lines	11, 14, footnote [f]
1.21J	Mac-1	NR[b]	NR[b]	NR[b]	94,000 180,000			
M3/38 M3/31	Mac-2	HLK,HL[a] HLK[a]	IgG_{2a} IgM	NR[b]	32,000	J774 macrophagelike line		3
M3/84	Mac-3	HL[a]	IgG_1	NR[b]	110,000	TG^C macrophages	Resident peritoneal macrophages, bone marrow cells, lymph node and spleen cells, thymocytes	2, 14, 15 f
54-2	Mac-4	NR[b]	IgG_{2a}	NR[b]	NR[d]	TG^C macrophages	Bone marrow cells, lymph node and spleen cells, thymocytes	2, 14
M3/37	Mac-4	NR[b]	NR[b]	NR[b]	180,000	Cultured bone marrow macrophages, TG^C macrophages, mast cells	Spleen, lymph node, thymus, bone marrow cells, neutrophils, resident peritoneal and alveolar macrophages, blood monocytes	16, 17
F480					160,000			
2.4G2	Fc receptor II		IgG		47,000-70,000[e]	TG^C macrophages	Bone marrow cells, lymph node and spleen cells, thymocytes	15
						Blood monocytes, resident and induced macrophages, J774, P815	Lymphocytes	19
						Macrophages, B lymphocytes	T lymphocytes	5, 18

[a] H and L, specific heavy and light chains; K, myeloma Kappa chain.
[b] NR: Not reported.
[c] Thioglycollate-induced.
[d] 54-2 and M3/37 precipitate polypeptides which coelectrophorese (M. Ho, unpublished).
[e] Depending upon the cell population.
[f] M. K. Ho and T. Springer. Mac-2, a novel 32,000 M_r macrophage subpopulation-specific antigen defined by monoclonal antibody, manuscript in preparation.

TABLE II. Monoclonal antibodies defining human monocytic differentiation antigens

Monoclonal antibody	Antigen designation	Subclass	Lysis	Antigen polypeptide chain	Tissue distribution: Positive	Tissue distribution: Negative	Functional studies	Reference
OKM1[a]	OKM1	IgG_{2b}	+	NR[b]	Blood monocytes, granulocytes, null cells, acute myelomonocytic L[c]	B and T lines, T, B-ALL, T, B-CLL, K562, HL-60	Proliferation to antigen	20
M1/70[d]	Mac-1	IgG_{2b}	NR[b]	NR[b]	Blood monocytes, granulocytes, NK, ADCC effectors		Natural killing, ADCC[e]	6
anti-Mol[a]	Mol	IgM	+	NR[b]	Blood monocytes, granulocytes, null cells, monocytic L, 21% bone marrow	K562, HL-60, U937		21
anti-Mo2[a]	Mo2	IgM	+	NR[b]	Blood monocytes, 11% bone marrow, monocytic L, U937 weak	Granulocytes, B and T lymphocytes, B and T lines, K562, HL-60		21
Mac-120[a]	Mac-120	NR[b]	+	120,000 M_r	30% Blood monocytes	B and T lymphocytes, neutrophils, HL-60, T and B lines	Proliferation to Con A and antigen, lymphokine production	10
63D3[a]		IgG_1	NR[b]	200,000 M_r	Blood monocytes, granulocytes weak	B lymphocytes? T lymphocytes, endothelial cells, lymphoblastoid lines, HL-60, U937		22
NR[b]					Spreading monocyte fibrils, neuronal cell processes	Blood monocytes		23

[a] Mouse antibody.
[b] NR: Not reported.
[c] L: Leukemia
[d] Rat antimouse antibody, cross-reactive with human cells.
[e] ADCC: Antibody-dependent cellular cytotoxicity.

phocytes except 2.4G2, directed to the Fc receptor II, which is expressed on B but not on T lymphocytes. The anti-Mac-1 MAb (M1/70) cross-reacts with both mouse and human macrophages. Mac-1 in the mouse and human and OKM1 and Mol in the human show highly similar expression on macrophages, granulocytes, and null cells, suggesting they may define homologous antigens. Several MAb define macrophage subpopulations, including M3/38 and 54-2 in the mouse and Mac-120 in the human. The Mac-4 antigen defined by 54-2 is expressed on both macrophage subpopulations and on mast cells. As is illustrated also by the pattern of expression of the Fc receptor II (Table I) and of 'jumping' or heterohistophile antigens such as Thy-1 (2), the sharing of a single antigen by two different cell types does not necessarily connote close ontogenetic relationship. Other MAb to murine macrophages that immunoprecipitate polypeptides of 82,000, 42,000, or 20,000 have been described in relation to their use for the study of the composition of pinocytic vesicles (3).

The ability to obtain large quantities of monospecific antibodies after growth *in vivo* or *in vitro* is a great advantage of hybridoma lines. The M1/70 line is currently available from the Cell Distribution Center, Salk Institute, P.O. Box 1809, San Diego, California 92112, and other lines should be available in the future. Since some lines can undergo chromosome losses leading to loss of antibody secretion (4), it is good practice to freeze aliquots of a line soon after receipt and to check specific antibody secretion after more than 6 months of growth in culture. Mouse - mouse or mouse - rat hybrids can be grown in syngeneic or irradiated hosts (5), respectively, to obtain ascites antibody, or *in vitro* to obtain 20 - 200 μg antibody/ml (2, 4). Methods for purifying MAb (6, 7) have been described.

Differencesin the use of MAb and classical sera relate to the homogeneity of MAb in both affinity and subclass. Some MAb have low affinity such that in the concentration ranges normally used ($\sim 10^{-9}$ - 10^{-6} *M*), the IgG or $F(ab')_2$ fragments will bind to cell surfaces (bivalent interaction), but Fab' fragments will not bind to cell surfaces nor will IgG immunoprecipitate monovalent antigen (monovalent interaction). M1/70 is an example of an MAb which can bind monovalently to the homologous mouse antigen but only bivalently to the cross-reacting human antigen (6).

MAb vary from excellent to poor in complement-mediated lysis (Tables I and II), depending on subclass. Lytic efficiency of the different rat MAb subclasses has been reported elsewhere (2, 8). Synergy for complement-mediated lysis between 2 MAb binding to different sites on the same surface molecule has been reported (9). Second-layer anti-Ig reagents have been used with the Mac-120 MAb to increase lytic efficiency (10).

Most studies with mouse MAb have relied more heavily on immunofluorescence than on complement-mediated lysis. Immunofluorescent labeling with MAb is far cleaner than with conventional sera, and has allowed the full resolving power of the fluorescence activated cell sorter to be realized (2). For rat anti-mouse MAb, both FITC-labeled SJL mouse anti-rat IgG (8) and mouse IgG-absorbed rabbit anti-rat IgG (11) have been used as second reagents. Immunofluorescent staining of thin sections with M1/70 MAb has recently been used to study the distribution of macrophages in spleen.[1]

Another difference between classical and MAb is that many hybrid lines secrete a mixture of hybrid molecules containing myeloma and specific chains. For example, an HLK line, i.e., secreting specific heavy (H) and light (L) and myeloma kappa (K) chains, would theoretically make only 25% of bivalently active H_2L_2 antibodies. In actuality, L and K chain secretion is often imbalanced (4) and thus the percentage of H_2L_2 molecules may considerably vary.

Purified MAb directly labeled with ^{125}I exhibit extraordinary specificity in binding assays (6, 12), particularly when antibody secreted by H_2L_2 variant clones (4) is used. Indirect binding assays with anti-Ig (2, 12) or *S. aureus* protein A (13) are also useful. In the case of *S. aureus* protein A, mouse IgG subclasses 1, 2a, and 2b (7), but only rat IgG 1 and 2c subclasses are reactive (2, 8).

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REFERENCES

1. G. Köhler and C. Milstein. Derivation of specific antibody-producing tissue culture and tumor lines by cell fusion. *Eur. J. Immunol. 6*: 511-519, 1976.
2. T. A. Springer. Cell-surface differentiation in the mouse. Characterization of "jumping" and "lineage" anti-

[1] *M. K. Ho and T. A. Springer. Rat anti-mouse macrophage monoclonal antibodies and their use in immunofluorescent studi of macrophages in tissue sections. In "Monoclonal Antibodies and T Cell Hybridomas: (U. Hammerling, G. Hammerling, and J. Kearney, eds.). Elsevier, New York, 1981.*

gens using xenogeneic rat monoclonal antibodies. *In* "Monoclonal Antibodies: (R. H. Kennett, T. J. McKearn, and K. B. Bechtol, eds.), pp. 185-217. Plenum, New York, 1980.

3. I. S. Mellman, R. M. Steinman, J. C. Unkeless, and Z. A. Cohn. Selective iodination and polypeptide composition of pinocytic vesicles. *J. Cell. Biol. 86*: 712-722, 1980.
4. T. A. Springer. Quantitation of light chain synthesis in myeloma x spleen cell hybrids and identification of myeloma chain loss variants using radioimmunoassay. *J. Immunol. Methods 37*: 139-152, 1980.
5. J. Unkeless. Characterization of a monoclonal antibody directed against mouse macrophage and lymphocyte Fc receptors. *J. Exp. Med. 150*: 580-596, 1979.
6. K. A. Ault and T. A. Springer. Cross-reaction of a rat-antimouse phagocyte-specific monoclonal antibody (anti-Mac-1) with human monocytes and natural killer cells. *J. Immunol. 126*: 359-364, 1980.
7. P. L. Ey, S. J. Prowse, and C. R. Jenkin. Isolation of pure IgG_1, IgG_{2a}, and IgG_{2b} immunoglobulins from mouse serum using protein A-sepharose. *Immunochemistry 15*: 429-436, 1978.
8. J. A. Ledbetter and L. A. Herzenberg. Xenogeneic monoclonal antibodies to mouse lymphoid differentiation antigens. *Immunol. Rev. 47*: 63-89, 1979.
9. J. C. Howard, G. W. Butcher, G. Galfre, C. Milstein, and C. P. Milstein. Monoclonal antibodies as tools to analyse the serological and genetic complexities of major transplantation antigens. *Immunol. Rev. 47*: 139-174, 1979.
10. H. V. Raff, L. J. Picker, and J. D. Stobo. Macrophage heterogeneity in man. A subpopulation of HLA-DR bearing macrophages required for antigen-induced T cell activation also contains stimulators for autologous-reactive T cells. *J. Exp. Med. 152*: 581-593, 1980.
11. T. Springer, G. Galfre, D. S. Secher, and C. Milstein. Mac-1: A macrophage differentiation antigen identified by monoclonal antibody. *Eur. J. Immunol. 9*: 301-306, 1979.
12. D. W. Mason and A. F. Williams. The kinetics of antibody binding to membrane antigens in solution and at the cell surface. *Biochem. J. 187*: 1-20, 1980.
13. G. Dorval, K. I. Welsh, and H. Wigzell. A radioimmunoassay of cellular surface antigens on living cells using iodinated soluble protein A from staphylococcus aureus. *J. Immunol. Methods 7*: 237-250, 1974.
14. T. A. Springer. Mac-1,2,3, and 4: Murine macrophage differentiation antigens identified by monoclonal antibodies. *In* "Heterogeneity of Mononuclear Phagocytes" (O. Förster, ed.). Academic Press, New York 1981 in press.

15. T. Springer. Monoclonal antibody analysis of complex biological systems: Combination of cell hybridization and immunoadsorbents in a novel cascade procedure and its application to the macrophage cell surface. *J. Biol. Chem. 256*: 3833-3839, 1981.
16. P. A. Leblanc, H. R. Katz, and S. W. Russell. A discrete population of mononuclear phagocytes detected by monoclonal antibody. *Infect. Immun. 8*: 520-525, 1980.
17. H. R. Katz, P. A. LeBlanc, and S. W. Russell. Antigenic determinant on mouse peritoneal mast cells. *J. Reticuloendothel. Soc.*, in press, 1981.
18. I. S. Mellman and J. C. Unkeless. Purification of a functional mouse Fc receptor through the use of monoclonal antibody. *J. Exp. Med. 152*: 1048-1069, 1980.
19. J. Austyn and S. Gordon. F4/80 - A specific rat monoclonal antimouse macrophage antibody. *In* "Heterogeneity of Mononuclear Phagocytes" (O. Forster, ed.). Academic Press, New York, 1981, in press.
20. J. Breard, E. L. Reinherz, P. C. Kung, G. Goldstein, and S. F. Schlossman. A monoclonal antibody reactive with human peripheral blood monocytes. *J. Immunol. 124*: 1943-1948, 1980.
21. R. F. Todd, III, L. M. Nadler, and S. F. Schlossman. Antigens on human monocytes identified by monoclonal antibodies. *J. Immunol. 126*: 1435-1442, 1981.
22. V. Ugolini, G. Nunez, R. G. Smith, P. Stastny, and J. D. Capra. Initial characterization of monoclonal antibodies against human monocytes. *Proc. Nat. Acad. Sci. USA 77*: 6764-6768, 1980.
23. N. Hogg and M. Slusarenko. Monoclonal antibodies to subsets of human blood monocytes. *Fourth Intl. Cong. Immunol. Abst.* 11.1.08, 1980.