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Chapter 1

Analysis of Macrophage Differentiation and Function with Monoclonal Antibodies

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I. INTRODUCTION

A large number of anti-mouse and anti-human macrophage/monocyte monoclonal antibodies (MAb) have recently been obtained that are proving invaluable reagents of extraordinary specificity for the study of macrophage differentiation, function, and surface antigen structure. This chapter summarizes information on such MAb reported up to February 1983 in tabular form, and then concentrates in more detail on antigens characterized by the authors and their collaborators.

In the mouse, at least five antigens have been found that can be distinguished by molecular weight, which are present on macrophages but not on lymphocytes (Table I). One of the mouse Fc receptors, present on macrophages as well as on some lymphocytes, has also been defined with MAb. Further antigens have not been defined biochemically, but appear to have distinct distributions on functional subpopulations.

Table I. Murine Macrophage Antigens Defined by Monoclonal Antibodies[a]

Antibody/antigen designation	Antigen polypeptide (MW)	Cellular distribution	Distinguishing features, functional features	Reference
Mac-1	95,000 170,000	Resident and exudative peritoneal macrophages, splenic histiocytes, granulocytes, PB monocytes, NK cells	Blocks type 3 complement receptor; absent from Langerhans, interdigitating, and lymphoid dendritic cells; M1/70 MAb binds to human PB monocytes, PMN, NK cells; 170,000-MW chain bears epitope	Springer *et al.* (1979), Mellman *et al.* (1980), Ault and Springer (1981), Ho and Springer (1982*b*), Holmberg *et al.* (1981), Beller *et al.* (1982)
Mac-2	32,000	TG-peritoneal exudative macrophages; weak or absent from resident and *Listeria*-exudative macrophages	Present on Langerhans and interdigitating, but not follicular dendritic cells; present on epithelial cells; absent from granulocytes	Springer (1981), Ho and Springer (1982*a*), Flotte *et al.* (1984), Haines *et al.* (1983)
Mac-3	110,000	Macrophages, granulocytes	Present on Langerhans and interdigitating, but not follicular dendritic cells; present on epithelial and endothelial cells	Springer (1981), Ho and Springer (1983*a*), Flotte *et al.* (1984), Haines *et al.* (1983)

54-2	180,000	Cultured bone marrow macrophages, TG-peritoneal exudative macrophages, mast cells	Absent from PB monocytes and resident macrophages	Leblanc *et al.* (1980), Katz *et al.* (1981)
F4/80	160,000	PB monocytes, resident and induced macrophages	Present on 8% bone marrow cells and on P815; macrophage activation reduces expression; 75,000-MW trypsin fragment bears epitope	Austyn and Gordon (1981), Hirsch *et al.* (1981), Ezekowitz *et al.* (1981)
2.4G2	47,000–70,000	Macrophages, PMN, B cells, J774, FcR-bearing T cells	Blocks binding of IgGl and IgG2b Fc to FcRII; protease resistant	Unkeless (1979), Mellman and Unkeless (1980)
ACM.1		Peritoneal macrophages activated by pyran or *Corynebacterium parvum;* absent from resident and protease exudative peritoneal macrophages	Cytotoxic for tumoricidal macrophage effectors	Taniyama and Watanàbe (1982)
M43, M57, M102, M143		Bone marrow macrophages, resident and exudative peritoneal macrophages	Define different cytotoxic macrophage subpopulations	Sun and Lohmann-Matthes (1982)

[a]*Abbreviations:* FcR, Fc receptor; MW, molecular weight; NK, natural killer; PB, peripheral blood; PMN, polymorphonuclear leukocytes; TG, thioglycollate.

A large number of human antigens have been characterized that are present on blood monocytes but that are present on none or on a small percentage of blood lymphocytes (Table II). In cases in which the antigens have been biochemically characterized, polypeptide chain molecular weights are indicated in brackets. Unfortunately, there has been little comparison of the anti-human MAb produced by different laboratories, and no doubt many of them identify the same antigens.

II. MAC-1 ANTIGEN

Mac-1 was the first antigen to be defined by MAb that is present on macrophages and not on lymphocytes (Springer *et al.*, 1979). The Mac-1 antigen expressed on myeloid but not on lymphoid cells. More recently, several other MAb to it have been obtained (Mellman *et al.*, 1980; Springer *et al.*, 1982; Sanchez-Madrid *et al.*, 1983*b*). The Mac-1 antigen contains two subunits, an α-subunit of 170,000 and a β-subunit of 95,000 relative molecular weight (M_r). The α- and β-subunits are not linked by disulfide bonds, but are tightly noncovalently associated in an $\alpha_1\beta_1$ complex (Kürzinger *et al.*, 1982; Kürzinger and Springer, 1982). Antibodies specific for the Mac-1 antigen bind to the α-subunit (Sanchez-Madrid *et al.*, 1983*b*). The LFA-1 antigen on lymphocytes has a different α-subunit associated with the same β-subunit, and thus antibodies to the β-subunit have a broader pattern of cellular reactivity. Both the α- and β-subunits are glycosylated and have surface exposure (Kürzinger and Springer, 1982). After digestion of intact cells with trypsin and papain, the α- and β-chains are cleaved, but remain bound to the cell surface and remain associated as shown by immunoprecipitation with both anti-α- and anti-β-MAb (P. Simon and T. A. Springer, unpublished observations). The subunits thus appear tightly associated with each other and with the cell membrane. Biosynthesis experiments have shown that the α- and β-subunits are derived from separate precursors of 161,000 and 87,000 M_r, respectively (Ho and Springer, 1983*b*). Assembly into the $\alpha_1\beta_1$ complex appears to precede processing of the subunits to their mature molecular weight, which presumably involves changes in glycosylation.

Mac-1 appears to be a universal macrophage marker (Ho and Springer, 1982*b*). It is expressed on >95% of peritoneal resident macrophages and macrophages elicited by thioglycollate, lipopolysaccharide (LPS), peptone, *Listeria monocytogenes*, and concanavalin A (Con A). The average amount of Mac-1 expressed per cell varies by no more than 2-fold among those different populations. Correlating with their larger size, thioglycollate-elicited macrophages express the highest number of anti-Mac-1 MAb binding sites per cell, 1.6×10^5. Mac-1 is present on splenic macrophages in the red pulp and in the marginal zones sur-

rounding the periarteriolar lymphoid sheath (Ho and Springer, 1982*b*) as well as on lymph node medullary cord macrophages (Flotte *et al.*, 1983). Histiocytes in the lamina propria of the intestine and alveolar macrophages in the lung are positive. Kupffer cells, which are distinct from macrophages but are in the mononuclear phagocyte lineage, are negative (Flotte *et al.*, 1983).

The Mac-1 antigen is also present on exudate granulocytes and granulocytic precursors in the bone marrow (Springer *et al.*, 1979) and on natural killer (NK) cells. Immunofluorescent cell sorter experiments have shown that cells with natural killing activity obtained from the nylon wool nonadherent fraction of peritoneal exudates are Mac-1$^+$ (Holmberg *et al.*, 1981). Furthermore, these Mac-1$^+$ cells have the morphology of large granular lymphocytes. The M1/70 rat anti-mouse MAb cross-reacts with human Mac-1, which has the same distribution as murine Mac-1, i.e., on monocytes, granulocytes, and null or large granular lymphocytes, which have NK and antibody-dependent cytotoxic activity (Ault and Springer, 1981). Human Mac-1 is equivalent to the OKM1 and Mo1 antigens (Todd *et al.*, 1982). The human granulocyte-monocyte precursor cell (CFUgm) appears to be Mac-1$^-$ (Smith *et al.*, 1984), Mol$^-$ (Griffin *et al.*, 1982). Thus, granulocytes and monocyte/macrophages become Mac-1$^+$ after divergence from their common stem cell. NK cells are renewed from the bone marrow (Kiessling *et al.*, 1977), but their stem cell has not been identified. It appears that there are at least two phenotypically distinct types of NK cells. NK cells (LGL) isolated directly from blood are mostly Mac-1$^+$ and OKM1$^+$ and mostly lacking in antigens characteristic of T cells (Ault and Springer, 1981; Ortaldo *et al.*, 1981; Breard *et al.*, 1981; Zarling *et al.*, 1981), while alloactivated or "cultured" NK cells express some T-cell markers and may be either Mac1/OKM1 positive (Krensky *et al.*, 1982) or negative (Brooks *et al.*, 1982; Sheehy *et al.*, 1983; Hercend *et al.*, 1983).

Mac-1 distribution on tumor cells in the monocyte/macrophage lineage parallels that on normal cells (Ralph *et al.*, 1983*a*). The immature myelomonocytic leukemia cell line M1 is Mac-1$^-$, but becomes strongly Mac-1$^+$ after induction with phorbol ester or lymphokines. Induced cells stop dividing and acquire functional properties and surface markers such as Fc and complement receptors characteristic of mature macrophages. In a study of eight independent macrophage lines ranging in phenotype from mature (J774 and P388D$_1$) to somewhat less mature (WEHI-3), all were found to be Mac-1$^+$. Cells of other hematopoietic lineages, including the P815 mastocytoma cell, were found to be Mac-1$^-$.

Monoclonal antibody blocking studies suggest an association or identity between Mac-1 and the complement receptor type 3 (CR$_3$), which is specific for C3bi (Beller *et al.*, 1982). The M1/70 anti-Mac-1 MAb strongly inhibits complement receptor-mediated rosetting of erythrocyte-IgM antibody-complement (E-IgM-C) complexes. Lack of inhibition by a panel of eight other antibodies, including anti-Mac-2, anti-Mac-3, and anti-H-2, and anti-panleukocyte MAbs which bind to a similar number of sites per cell as anti-Mac-1, demonstrates the

Table II. Human Monocyte/Macrophage Antigen Expression on Cells within the Myeloid Lineage[a, b]

Bone marrow

Erythroid precursors	Monocytic precursors		Granulocyte-monocyte stem cell	Granulocytic precursors		Megakaryocyte
				Early	Late	
5F1	Mo1	My8	My7	Mo5	3G8	Mo4
	Mo2	B13.4	D5D6	My8	Mo1	MPA
	Mo5	5F1	C10H5	My7	Mo5	
	My3	1G10		1G10	Mo6	
	My4				My8	
	My7				B13.4	
					B43.4	
					1G10	

Peripheral blood

Monocyte[c-f]			Granulocyte[e]		Platelet
Mac-120[120] (1)	1D5 (12)	B13.4 (7)	3G8	AML-2-23	Mo4
Mo2[55] (2–4)	S4-7 (13)	B43.4 (7)	OKM1	1G10	63D3
Mo3 (4, 5)	63D3[200] (6, 14)	B34.3 (7)	Mo1	S5-7	5F1
Mo4 (4, 5)	OKM1[94, 155] (15)	5F1 (24)	Mac-1	MMA	MPA
61D3[23, 55] (6)	Mo1[94, 155] (2–4)	AML-2-23 (25)	B2.12	*My4*	
B44.1 (7)	Mac-1 (16)	MPA[93, 135] (26)	M522	*63D3*	
D5D6 (8)	My903 (17)	MφP-9 (9)	Mo5	*Mo6*	
C10H5 (8)	My904 (17)	MφS-1 (9)	My7	*My3*	
MφP-15 (9)	B2.12 (18)	MφS-39 (9)	My8	*MφS-39*	
MφP-7 (9)	Mo6[80] (4, 19)	*1G10* (24)	B13.4	*MφP-9*	
MφR-17 (9)	M522 (20)	*My7[160]* (21)	B43.4	*MφS-1*	
PHM2 (10)	My3 (21)	UC45[45] (27)	B34.3	*S4-7*	
PHM3 (10)	My4[55] (21)	S5-25 (13)		*S5-25*	

OKM5 (11)	My8 (21)	S5-7 (13)
	4F2[40, 80] (22)	
	MMA (23)	

Extravascular space macrophage[c,d]	
3G8[47–70] (28)	
Mo1	MφS-1
Mo2	MφS-39
Mo4	MφP-15
MφP-9	MφP-7
ID5	MφR-17
PHM2	PHM3

[a]Modified from Todd *et al.* (1983).
[b]Almost all the monoclonal antibodies have been tested on erythrocytes, monocytes, granulocytes, and lymphocytes, but many have not been tested on bone marrow cells, macrophages, and nonhematopoietic cells. A few of the antibodies such as PHM2 and 4F2 react with small percentages of resting lymphocytes or with activated lymphocytes.
[c]M_r of polypeptide chains $\times 10^{-3}$ is in brackets.
[d]Underline indicates specificity for monocytes or macrophages (among hematopoietic cells).
[e]Italics indicate weak expression.
[f]Numbers in parentheses are keyed to the following references:

1. Raff *et al.* (1980)
2. Todd *et al.* (1981)
3. Todd *et al.* (1982)
4. Todd *et al.* (1984)
5. Todd and Schlossman (1982)
6. Ugolini *et al.* (1980)
7. Perussia *et al.* (1982)
8. Linker-Israeli *et al.* (1981)
9. Dimitriu-Bona *et al.* (1983)
10. Becker *et al.* (1981)
11. Shen *et al.* (1983)
12. Kaplan and Gaudernack (1982)
13. Ferrero *et al.* (1983)
14. Rosenberg *et al.* (1981)
15. Breard *et al.* (1980)
16. Ault and Springer (1981)
17. Letvin *et al.* (1983)
18. Van Der Reijden *et al.* (1983)
19. Todd and Schlossman (1984)
20. Lohmeyer *et al.* (1981)
21. Griffin *et al.* (1981)
22. Haynes *et al.* (1981)
23. Hanjan *et al.* (1982)
24. Bernstein *et al.* (1982)
25. Ball *et al.* (1982)
26. Burckhardt *et al.* (1982)
27. Hogg (1983)
28. Fleit *et al.* (1982)

specificity of blockade. Inhibition occurs with as little as 1 μg/ml of anti-Mac-1 $F(ab')_2$ fragments, and the Fc receptor is unaffected. Macrophages bear receptors for both C3b (CR_1) and C3bi (CR_3). Primarily the latter receptor is measured when E are sensitized with C5-deficient serum (Ross, 1980). When E bearing only C3b or C3bi were prepared with homogeneous complement components, it was found that Mac-1 inhibited the CR_3 but not the CR_1 (Beller *et al.*, 1982). Since the M1/70 anti-Mac-1 MAb is cross-reactive with human cells, it was also tested for its ability to inhibit complement receptors on human cells. Anti-Mac-1 inhibits the CR_3 but not the CR_1 on human granulocytes. The most likely interpretation of these findings is that Mac-1 antigen is the CR_3. Studies with monoclonal antibodies to the human OKM1 antigen, which appears identical to human Mac-1, lend further support to this idea (Wright *et al.*, 1983). *Staphylococcus aureus* bacteria coated with a sandwich of OKM1 antibody and OKM1 antigen specifically agglutinate with C3bi-coated E. Since the CR_3 does not have sufficiently high affinity to bind soluble C3bi, it has not been possible to test for displacement of soluble ligand.

It is interesting to compare the distribution of Mac-1 with that reported for the CR_3 (reviewed in Ross, 1980; Fearon and Wong, 1983). The CR_3 is present on monocytes and neutrophils, as is Mac-1. It also is present on 6–10% of human blood lymphocytes (Ross and Lambris, 1982; Perlman *et al.*, 1981), in agreement with the finding of Mac-1 on the null subpopulation of ~10% of human lymphocytes that contain NK activity (Ault and Springer, 1981). There are conflicting findings on the presence of the CR_3 on glomerular epithelial cells (Carlo *et al.*, 1979; Beller *et al.*, 1982); kidney is negative for Mac-1 by absorption and thin section staining. B-lymphoblastoid cell lines and less than one-third of tonsil lymphocytes have been reported to express the CR_3 (Ross and Lambris, 1982). However, B cells also express the complement receptors CR_2 and CR_1. The CR_2 binds C3bi in addition to C3d; since antibodies to the CR_2 largely inhibited rosetting with E-C3bi by B lymphoblastoid lines, it is possible that this rosetting is caused by the CR_2. Furthermore, although the CR_1 is specific for C3b at physiologic ionic strength, it can bind C3bi under the low-ionic-strength conditions sometimes used in complement adherence assays (Ross *et al.*, 1983).

III. THE MAC-1 AND LFA-1 FAMILY

A second antigen has been discovered that is distinct from Mac-1 in cell distribution, function, and α-subunit structure, but appears to use the same β-subunit. In the course of studies on the molecular basis of T-cell function, MAb were selected for their ability to inhibit antigen-specific T-lymphocyte-mediated killing (Springer *et al.*, 1982). Some of these MAb defined the LFA-1 antigen,

which contains two polypeptide chains of M_r 180,000 and 95,000. MAb to LFA-1 block killing by inhibiting formation of the adhesion between the cytolytic T lymphocyte (CTL) and the target cell (Davignon *et al.*, 1981; Springer *et al.*, 1982). It appears that LFA-1 is distinct from the antigen receptor, but works together with it in contributing to the avidity of the CTL for the target cell (Springer *et al.*, 1982). LFA-1 is present on B lymphocytes and myeloid cells as well as T lymphocytes (Kürzinger *et al.*, 1981), suggesting that it plays a more general role in adhesion than do antigen receptors. Interestingly, both adhesion of CTL to target cells, the step in which LFA-1 participates (Springer *et al.*, 1982), and adhesion of macrophages to C3bi-coated cells, which is mediated by the CR_3, are Mg^{+2}-dependent (Lay and Nussenzweig, 1968; Wright and Silverstein, 1982).

The Mac-1 and LFA-1 β-subunits of M_r 95,000 are identical by peptide mapping and by complete immunologic cross-reactivity (Kürzinger *et al.*, 1982; Sanchez-Madrid *et al.*, 1983*b*). Similar peptide map results were obtained for Mac-1 and an antigen probably identical to LFA-1 (Trowbridge and Omary, 1981). A MAb cross-reactive for Mac-1 and LFA-1 has been shown to bind to an epitope on their β-subunits (Sanchez-Madrid *et al.*, 1983*b*). MAb, which are specific for Mac-1 or LFA-1, bind to α-chain epitopes. The α-subunits are non-cross-reactive, as shown with both monoclonal and conventional antisera, and have different tryptic peptide maps. However, sequencing of their N termini has shown 40% amino acid sequence homology (D. Teplow, W. Dreyer, and T. Springer, unpublished data), suggesting that the α-chains are related by gene duplication.

MAb binding to distinct topographic sites on Mac-1 and LFA-1 have been shown to differ in their functional effects (Sanchez-Madrid *et al.*, 1983*b*). Two MAb recognizing closely related topographic determinants on the Mac-1 α-chain inhibit complement receptor activity, while a third anti-α-chain MAb directed against a topographically distinct α-determinant and an anti-β-MAb have no effect. In fact, the anti-β-MAb consistently enhances CR_3, activity. These results indicate that a functionally active site on the Mac-1 molecule, probably representing the ligand binding site, can be localized to a specific region of the α-chain. Similarly, a functionally active site on the LFA-1 molecule has been localized to the α-subunit.

A similar family of related molecules has been found on human cells (Sanchez-Madrid *et al.*, 1983*a*). Human Mac-1 is homologous to mouse Mac-1, as shown by monoclonal antibody cross-reaction, identical cell distribution of the antigens (Ault and Springer, 1981), and identical association with the CR_3 (Beller *et al.*, 1982). Human Mac-1 appears identical to the human OKM1 and Mo1 antigens in terms of cell distribution, and anti-OKM1 and anti-Mo1 MAb also block complement receptor activity (Todd *et al.*, 1984; Wright *et al.*, 1983; and Sanchez-Madrid *et al.*, 1983*a*). Human LFA-1 is equivalent to mouse LFA-1 and shares a common β-subunit with OKM1 (Sanchez-Madrid *et al.*, 1983*a*).

Furthermore, yet a third antigen with a distinct α-chain of 150,000 M_r has been found to be associated with the same β-subunit. It is found on granulocytes and monocytes (Sanchez-Madrid *et al.*, 1983*a*). Thus, an interrelated family of three different cell-surface molecules has been described which use a single type of β-subunit in association with differing α-subunits. Two of these molecules are associated with cell-adhesion functions, and it will be interesting to determine whether the third has a similar function.

IV. MAC-2 ANTIGEN

Mac-2 is a macrophage surface antigen of 32,000 M_r. It is biosynthesized by macrophages (Ho and Springer, 1982*a*), and the precursor identified by 5-min pulse labeling is the same molecular weight as the mature antigen (M. K. Ho and T. A. Springer, unpublished observations). Isoelectric focussing shows that Mac-2 is a basic polypeptide having a pI in the range of 7–8. It focuses in a position very close to that of the invariant chain of Ia, which is of 31,000 M_r (Jones *et al.*, 1979). However, there are no identities between the methionyl tryptic peptides of Mac-2 and the Ia invariant chain (M. K. Ho and T. A. Springer, unpublished data). Thioglycollate-elicited macrophages bear 1.7×10^5 anti-Mac-2 MAb binding sites per cell.

Mac-2 is a macrophage subpopulation marker, an inducible component of the macrophage cell surface (Ho and Springer, 1982*a*). Among resident macrophages and five different types of elicited peritoneal macrophages studied, only thioglycollate-elicited macrophages showed strong expression by immunofluorescent flow cytometry and immunoprecipitation of ^{35}S-methionine-labeled antigen. Mac-2 is expressed equally strongly by macrophages 1 day and 4 days after elicitation with thioglycollate. Thus, cells recruited into the peritoneum after 1 day are already committed to the synthesis of Mac-2. Biosynthesis of Mac-2 by resident peritoneal macrophages and macrophages elicited by peptone, LPS, Con A, and *Listeria* is detected, but is 10- to 30-fold lower than in thioglycollate-elicited macrophages. Mac-2 is essentially undetectable on these low-expressing cells by immunofluorescence, but is detectable by the much more sensitive immunoperoxidase technique (Flotte *et al.*, 1983). Mac-2 has been found on all mature macrophage cell lines examined and is absent from lymphoid and primitive erythroid and myelomonocytic lines. In contrast to Mac-1 and Mac-3, however, Mac-2 is not expressed by the M1 cell line after induction of maturation (Ralph *et al.*, 1983*a*).

Resident peritoneal macrophages or those elicited by a variety of agents synthesize and express on their surface similar amounts of the Mac-1 antigen (Ho and Springer, 1982*b*). Mac-1 is thus a constitutive macrophage marker, whereas Mac-2 and Ia (Beller *et al.*, 1980) appear to be inducible surface components.

The induction of Mac-2 and Ia is controlled independently, since some macrophages such as thioglycollate-elicited are high in Mac-2 and low in Ia, while *Listeria*-elicited macrophages are high in Ia and low in Mac-2. Resident macrophages are low in both antigens.

Immunoperoxidase shows that all tissue macrophages, such as alveolar macrophages and macrophages in the lamina propria are Mac-2^+, although less strongly than thioglycollate-elicited macrophages (Flotte *et al.*, 1983). Kupffer cells are also Mac-2^+. Surprisingly, Mac-2 is also expressed in a highly specific pattern on certain epithelial cells. It is present on bronchial epithelium, some kidney tubules, intestinal epithelium, in the skin on keratinocytes, hair follicles, and sweat ducts, and in the brain on the choroid plexus and ependyma. It appears that Mac-2 is induced during the maturation of intestinal epithelial cells. The epithelial cells of villous intestine are renewed in crypts just below the villi. They migrate from the villum base to the tip, from which they are eventually sloughed off. Crypt epithelial cells are Mac-2^-, those at the base of villi stain weakly, and there is a gradient of increasing Mac-2 expression from the base to the tip of villi. When intestinal epithelial cells are stained histochemically it is found that their ability to absorb nutrients such as fats from the lumen, follows a similar distribution (Ladman *et al.*, 1963).

Thus far, no functional activity of macrophages has been found to be inhibited by anti-Mac-2 MAb, including mannose uptake, which appears to be mediated by a receptor of similar molecular weight (Townsend and Stahl, 1981). Because thioglycollate-elicited macrophages are more active phagocytically than the other macrophages studied, and because certain epithelial cells are highly active endocytically, it is possible that Mac-2 plays a role in endocytosis.

V. MAC-3 ANTIGEN

Mac-3 (Ho and Springer, 1983*b*) is a less abundant antigen present in 3.6×10^4 sites per thioglycollate-elicited macrophages. It is expressed in similar quantities on resident peritoneal macrophages and on macrophages elicited by a variety of agents. It is found on macrophages in a number of tissues examined by immunoperoxidase staining of thin sections (Flotte *et al.*, 1983, 1984) and on eight of eight different macrophage cell lines (Ralph *et al.*, 1983*a*). Mac-3 is a glycoprotein and appears as a somewhat diffuse band in sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). Mac-3 is found on macrophages and granulocytes but on no other hematopoietic cell types. It is also found on some nonhematopoietic cell types, giving highly specific staining patterns on epithelial and endothelial cells in a variety of tissues (Flotte *et al.*, 1983). Both liver parenchymal and Kupffer cells are stained, and staining of bile canaliculi is partic-

ularly intense. Intestinal epithelial cells are stained only on their luminal border, perhaps on microvilli.

An unusual feature of Mac-3 is that its molecular weight varies from 100,000 to 170,000 depending on the type of elicited macrophage or macrophage cell line from which it is isolated (Ho and Springer, 1983*a*; Ralph *et al.*, 1983*a*). Macrophages elicited by different agents synthesize identical Mac-3 precursors of 74,000 M_r which are processed in 15 min to the higher-molecular-weight mature forms, which vary in M_r depending on the type of macrophage population (Ho and Springer, 1983*a*). This shift appears to be attributable to glycosylation. Recent studies of Mac-3 carbohydrate show that high-mannose, complex, and lactosaminoglycan moieties are present (A. Mercurio, P. Robbins, and T. Springer, unpublished results). It appears that Mac-3 is glycosylated to different extents in macrophages depending on their state of differentiation. Glycosylation may have important effects on the surface properties of macrophages and important consequences for macrophage functional activity. Such variation in glycosylation may thus be an important source of macrophage heterogeneity.

VI. LANGERHANS CELLS, DENDRITIC CELLS, AND MACROPHAGES

The requirement for accessory cells in the induction of antigen-specific T-lymphocyte responses is well documented. However, the relationship between different types of accessory cells has been unclear. Ia antigen-bearing macrophages are potent antigen-presenting cells (Unanue, 1981) and are probably particularly important at inflammatory sites. Langerhans cells are the antigen-presenting cells of the skin. They mediate the induction of contact sensitivity *in vivo* and the induction of antigen-specific T-lymphocyte responses *in vitro* (Silberberg-Sinakin *et al.*, 1976; Stingl *et al.*, 1978). Interdigitating dendritic cells are found in the T-dependent areas of lymphoid tissues. Their long dendritic processes, which resemble those of Langerhans cells, form extensive contacts with adjacent T lymphocytes in thymus, spleen, and lymph node (Thorbecke *et al.*, 1980; Tew *et al.*, 1982). This anatomic association and the morphologic resemblance to Langerhans cells including the sharing of the unique tennis racquet-shaped Birbeck granule suggest that interdigitating cells are important in antigen presentation and in regulating T-cell responses. Follicular dendritic cells, found in intimate contact with B lymphocytes in the corona of lymphoid follicles, differ in morphology from Langerhans and interdigitating dendritic cells. They take up antigen-antibody-complement complexes and retain them longer *in vivo* than any other cells (Thorbecke *et al.*, 1980; Humphrey and Grennan, 1984). The lymphoid dendritic cell of Steinman and Cohn (1973) has been isolated in suspension, has a dendritic morphology, and is an active ac-

cessory cell for lymphocyte responses. Its precise relationship to the interdigitating and follicular dendritic cells is unclear. Macrophages and Langerhans cells are bone marrow derived, but the relationship of their precursors is unknown (Stingl *et al.*, 1978).

The surface markers of dendritic cells and macrophages have been compared (Table III). All except follicular dendritic cells bear Ia antigen, which is important in the induction of antigen-specific T-lymphocyte responses (Germain *et al.*, 1982). Macrophages can be distinguished from all the other cell types by their expression of the Mac-1 antigen. The lack of expression of Mac-1 on Langerhans cells is in agreement with the absence of C3bi receptors (Berman and Gigli, 1980). The lymphoid dendritic cell of Steinman and Cohn also lacks Mac-1. Interestingly, Langerhans cells and interdigitiating cells express the Mac-2 and Mac-3 antigens, whereas follicular dendritic cells are negative for both antigens. The common Mac-$1^-2^+3^+$ phenotype of Langerhans cells and interdigitating dendritic cells supports the ideas, based on morphologic similarites, that these may be ontogenetically and functionally related cells localized in different anatomic

Table III. Properties of Murine Dendritic Cells and Macrophages[a, b]

Property/antigen	Macrophage[f]	Langerhans cells[f]	Interdigitating dendritic cell[f]	Follicular dendritic cell[f]	Lymphoid dendritic cell[f]
Ia	+/- (1)	+ (2)	+ (2)	- (3)	+ (4, 8)
Mac-1	+ (5)	- (6, 7)	- (6, 7)	- (6, 7)	- (8)
Mac-2	+ (9)	+ (6, 7)	+ (6, 7)	- (6, 7)	ND
Mac-3	+ (10)	+ (6, 7)	+ (6, 7)	- (6, 7)	ND
Ly-5/CLA	+ (5, 11)	+ (6, 7)	ND	ND	+ (8)
FcR	+ (12)	+ (6, 7, 13)	ND	+ (3)	- (8, 14)
C3b R	+ (15)	+ (16)[c]	ND	+? (3)[d]	-/+ (14)[d,e]

[a]Modified from Haines *et al.* (1983).
[b]The terminology is that of Tew *et al.* (1982). CLA, common leukocyte antigen; FcR, Fc receptor; ND, not determined.
[c]Langerhans cells bear the receptor for C3b and lack receptors for C3bi and C3d (Berman and Gigli, 1980).
[d]Results were reported for C3R, but it is not known whether C3b R, C3bi R, or both were measured.
[e]Mouse spleen dendritic cells are $C3R^-$, and human peripheral blood dendritic cells are $C3R^+$ (Van Voorhis *et al.*, 1982).
[f]Numbers in parentheses are keyed to references:

1. Swartz *et al.* (1976)
2. Hoffman-Fezer *et al.* (1978)
3. Humphrey and Grennan (1984)
4. Steinman *et al.* (1979)
5. Springer *et al.* (1979)
6. Flotte *et al.* (1983)
7. Haines *et al.* (1983)
8. Nussenzweig *et al.* (1981)
9. Ho and Springer (1982*a*)
10. Ho and Springer (1983*a*)
11. Scheid and Triglia (1979)
12. Berken and Benacerraf (1966)
13. Tamaki *et al.* (1979)
14. Steinman and Cohn (1974)
15. Fearon and Wong (1983)
16. Burke and Gigli (1980)

sites and that these cells differ from follicular dendritic cells. It will be interesting to learn whether lymphoid dendritic cells are also Mac-2^+3^+. All cell types tested, i.e., macrophages, Langerhans cells, and lymphoid dendritic cells, share the Ly-5 or common leukocyte antigen (CLA) molecule. This marker has thus far only been found on hematopoietic cells (Scheid and Triglia, 1979; Springer, 1980; Kürzinger *et al.*, 1981; Sarmiento *et al.*, 1982).

VII. DEFINING MACROPHAGES BY THEIR SURFACE MARKERS

The Mac-1 and Mac-3 surface markers are acquired during differentiation from immature precursor cells, as shown in mice (Ralph *et al.*, 1983*a*) and humans (Ralph *et al.*, 1983*b*) with the M1 and U937 tumor line models, respectively. Studies in humans have shown that the granulocyte-monocyte colony forming unit is Mac-1^- or low in Mac-1 (Griffin *et al.*, 1982; Smith *et al.*, 1983). Mac-1 expression is increased during differentiation of peripheral blood monocytes to peritoneal macrophages (Springer *et al.*, 1979). Mac-1 and Mac-3 have been found on all mature macrophages and histiocytes studied and thus may be considered constitutive markers of mature macrophages. The Mac-2 antigen is found in all types of macrophages, as shown by the immunoperoxidase technique, although it should be considered an inducible marker because of the wide variation in quantitative expression. Coexpression of the Mac-1 and Mac-2 antigens, with the caveat that the sensitive immunoperoxidase technique must be used to detect Mac-2, appears to be an excellent operational definition of the macrophage in the many different anatomic sites so far investigated. Mac-1 is also found on granulocytes and NK cells and Mac-2 is also found on epithelial, Langerhans, and interdigitating cells, but the markers are found together only on macrophages.

Few surface markers are specific for what hematologists would define as a cell lineage. This is not surprising, because structures are present on cell surfaces to perform specific functions, not for the convenience of hematologists or immunologists. The cells of the immune defense system appear to have evolved a high degree of functional redundance; e.g., cells of both the lymphoid and myeloid lineages bear Fc and C3b receptors for immune complexes and can phagocytose foreign material.

Many types of cells can act as accessory cells for the induction of antigen-specific T-lymphocyte responses, and the expression of Ia antigens is correspondingly widespread. Even skin epithelial cells become Ia^+ when the skin is inflamed in graft-versus-host reactions (Mason *et al.*, 1981), and endothelial cells become Ia^+ in response to γ-interferon secreted by lymphocytes (Pober and Gimbrone, 1982).

Markers may be useful in an operational sense for defining lineages, but their

use in predicting the relatedness of cells is limited, at best. For example, the 54-2 antigen is found on elicited and not on resident macrophages (Leblanc *et al.*, 1980), but also marks mast cells (Katz *et al.*, 1981). Mac-2 and Mac-3 are present on macrophages and not on lymphocytes, but are also present on epithelial, and for Mac-3, additional nonhematopoietic cells. The OKT6 antigen, originally thought to be thymocyte specific, has recently also been found on Langerhans cells (Fithian *et al.*, 1981). The Thy-1 antigen has long been used as a marker for distinguishing T lymphocytes from other cells of the hematopoietic system; however, it was only recently discovered to be present as well on 25% of bone marrow cells, including stem cells, and on myeloid cells in bone marrow cultures (Schrader *et al.*, 1982; Basch and Berman, 1982). In the definition of cell lineages, there appears to be no substitute for the direct study of stem cell development into mature cell types.

When the function of surface markers is known, their expression on diverse cell types takes on greater significance. The expression of Mac-1 on both macrophages and on NK/antibody-dependent cytotoxic cells is an example. On macrophages, the CR_3 (Mac-1) mediates adherence to cells or particles opsonized with the complement component C3bi. On activated macrophages, the CR_3 mediates phagocytosis (Michl *et al.*, 1979). On resident macrophages, the CR_3 is synergistic with the FcR for phagocytosis (Bianco and Nussenzweig, 1977). What is the role of the CR_3 (Mac-1) on NK and antibody-dependent cellular cytotoxicity (ADCC) cells, which are nonphagocytic? When target cells are coated with C3bi in addition to IgG, lysis by ADCC effectors is greatly enhanced (Perlman *et al.*, 1981), suggesting that the CR_3 synergizes the the Fc receptor (FcR) in the killing reaction. Whether the presence of C3bi on target cells would enhance natural killing has not yet been tested.

VIII. MOUSE $Fc_{\gamma 2b/\gamma 1}R$

The analysis of mouse FcR has been complicated by the apparent presence of several receptors with specificity for different subclasses of IgG. The FcR that binds mouse IgG2a ($Fc_{\gamma 2a}R$) is inactivated by trypsinization (Unkeless and Eisen, 1975; Walker, 1976), while those binding IgG2b and IgG1 ($Fc_{\gamma 2b/\gamma 1}R$) and IgG3 (Diamond and Yelton, 1981) are resistant to trypsin. Although the results of competition experiments with monomeric IgG myeloma proteins are equivocal with respect to FcR heterogeneity (Segal and Titus, 1978; Haeffner-Cavaillon *et al.*, 1979), competition experiments using aggregated IgG and immune complexes of different subclasses indicate that there are three different FcR on macrophages (Walker, 1976; Diamond and Scharff, 1980; Diamond and Yelton, 1981). Comparable results have been obtained in rats (Boltz-Nitulescu *et al.*, 1981). These conclusions about receptor heterogeneity are supported by

the isolation of macrophage cell line variants lacking the $Fc_{\gamma 2b/\gamma 1}R$ and the $FcR_{\gamma 3}$ receptor, respectively (Unkeless, 1979; Diamond and Yelton, 1981).

The mouse macrophage FcR specific for IgG2b and IgG1 immune complexes has been characterized using a rat monoclonal antibody, 2.4G2 (Unkeless, 1979; Mellman and Unkeless, 1980). The monoclonal antibody was isolated after the fusion of spleen cells from a rat immunized with the mouse macrophage cell lines J774 and $P388D_1$ and was identified by the ability of the culture cell supernatant to inhibit rosette formation with sheep erythrocytes (E) opsonized with monoclonal anti-E IgG2b immunoglobulin. The specificity of the monoclonal antibody 2.4G2 was examined by studying the inhibition, after preincubation of macrophages with the Fab fragment of 2.4G2, of rosette formation with opsonized erythrocytes. Only the binding of IgG2b and IgG1 immune-complex-coated E was inhibited. The binding of IgG2a-immune-complex-coated E was unaffected.

The cellular distribution of the antigen was determined by quantitative binding studies and by inhibition of rosette formation with E opsonized with rabbit IgG (EIgG). In addition to its presence on all mouse macrophages, the 2.4G2 antigen is present on monocytes, B lymphocytes, polymorphonuclear leukocytes (PMN), and several lymphoid cell lines of T-cell and null-cell origin. These results demonstrated the antigenic identity of FcR on a variety of cell types. The 2.4G2 determinant is, however, absent from mouse dendritic cells (Nussenzweig *et al.*, 1981).

The $Fc_{\gamma 2b/\gamma 1}R$ was purified by affinity chromatography on 2.4G2 Fab-Sepharose 4B (Mellman and Unkeless, 1980). Nonionic detergent lysates of J774 tumors or cultured J774 cells were absorbed on an affinity column, which was then washed with Nonidet® P-40 SDS-mixed micelles, followed by 0.5% sodium deoxycholate. The bound protein was then eluted with 0.5% sodium deoxycholate adjusted to pH 11.5 with triethylamine, following a procedure developed for purification of Ia antigens (McMaster and Williams, 1979). The protocol resulted in the isolation of 0.01% of the protein in the initial lysate after clearance of nuclei, and the recovery of 2.4G2 antigen was 57%, an overall purification of $>$ 5000-fold in one step.

The $Fc_{\gamma 2b/\gamma 1}R$ thus isolated from the mouse macrophage line J774 consists of two poorly resolved peptides of 47,000 and 60,000 M_r. The peptides are glycosylated and can be labeled by galactose oxidase oxidation followed by reduction with $NaB[^3H_4]$. In two-dimensional isoelectric focusing SDS-PAGE, the $Fc_{\gamma 2b/\gamma 1}R$ exhibits the typical decrease *in* M_r from acidic to basic species. The isoelectric point of the purified receptor is broad, with a pI of 4.7-5.8. This was later confirmed by Lane and Cooper (1982), who isolated FcR by affinity chromatography using both 2.4G2 IgG and affinity chromatography on IgG2b-Sepharose. Lane and Cooper (1982) also observed small differences in M_r and isoelectric point between Fc-binding proteins isolated from IgG2a-Sepharose compared with proteins eluted from IgG2b-Sepharose, suggesting that the two

receptors have structural differences. Others have isolated similar Fc-binding proteins (Loube *et al.*, 1978; Loube and Dorrington, 1980; Schneider *et al.*, 1981) from mouse macrophages or macrophage cell lines. However, differences between proteins isolated on IgG2a versus IgG2b or human IgG1-Sepharose were not detected in these studies.

The 2.4G2 antigen, and the activity of $Fc_{\gamma 2b/\gamma 1}R$ is trypsin resistant and can be solubilized from the plasma membrane only by detergents, indicating it is an integral membrane protein. However, trypsin treatment does result in a decrease in the amount of the higher M_r peptide, and a concomitant increase in the amount of the lower M_r peptide. This result suggests that the two peptides isolated from J774 cells may be related by a post-translational proteolytic event. The similarity between the typtic and chymotryptic maps of the two peptides is consistent with that interpretation (I. Mellman and J. Unkeless, unpublished data), although the possibility that two closely related peptides are translated from different messages is not ruled out. $Fc_{\gamma 2b/\gamma 1}R$ was immunoprecipitated from a variety of cell lines after surface iodination, and a significant variation in M_r was observed, with the largest species from the B-cell line WEHI-231 and the smallest from thioglycollate-elicited peritoneal macrophages. The biochemical basis for this variation and any functional correlates of these differences are unknown.

Of particular interest was the observation that the purified $Fc_{\gamma 2b/\gamma 1}R$ retained binding specificity for IgG consistent with identification of the protein as an FcR. Although there was no binding of the purified protein to $F(ab')_2$ immune-complex-coated surfaces, the specificity of binding to mouse IgG subclasses was partially lost. In the absence of detergent, the receptor bound to IgG2b-, IgG1-, and IgG2a-coated Sephadex beads, but not to IgG3-coated Sephadex beads. However, in the presence of detergent, purified and labeled $Fc_{\gamma 2b/\gamma 1}R$ bound best to IgG2b aggregates, less well to IgG1 aggregates, and not at all to either IgG2a or rabbit IgG (I. Mellman and J. Unkeless, unpublished data). The purified FcR in the absence of detergent formed aggregates of large size (*S* value: 15). We attribute the previously observed lack of specificity to the magnification of a low avidity of binding resulting from the multivalent nature of the receptor in the absence of detergent.

The interaction of the FcR on mouse macrophages with immune complexes results in the triggering of the cell's defense mechanisms, which range from phagocytosis of the offending particle to release of hydrolytic enzymes, superoxide, prostaglandins, and leukotrienes. The nature of the signal transmitted to the cell by the FcR was studied using the lipophilic tetraphenylphosphonium cation (TPP^+) as a probe of macrophage membrane potential (Lichtshtein *et al.*, 1979). The effect of exposure of J774 macrophagelike cells to immune complexes and the anti-$Fc_{\gamma 2b/\gamma 1}R$ monoclonal 2.4G2 as well as other monoclonal antibodies was tested (Young *et al.*, 1983*a*). The resting potential of cells of the J774 macrophage cell line determined from TPP^+ equilibration data was −15 mV.

Extensively cross-linked immune complexes or 2.4G2 Fab coupled to Sephadex beads presented to J774 cells resulted in a prompt depolarization that lasted 15-20 min. Soluble immune complexes or the bivalent 2.4G2 IgG resulted in a transient depolarization, followed by a hyperpolarization that was blocked by prior incubation with ouabain. The depolarization was due to an influx of Na^+, since replacement of Na^+ by choline, which did not affect the membrane potential, abolished the depolarization in response to immune complexes. Other monoclonal antibodies 2D2C, 2E2A, and 1.21J, all of which recognize major antigenic determinants on J774 cells (Mellman *et al.*, 1980; Nussenzweig *et al.*, 1981; Muller *et al.*, 1983) stimulated a ouabain-blockable hyperpolarization.

These results are compatible with the thesis that the FcR functions as a ligand-dependent ion channel. To investigate this possibility, the ion flux into plasma membrane vesicles isolated from J774 cells was examined by TPP^+ uptake after dilution of vesicles with entrapped cations into isotonic sucrose containing ligands and labeled TPP^+ (Young *et al.*, 1983*b*). In the presence of immune complexes or 2.4G2 IgG there was a prompt and substantial uptake of TPP^+ over that observed in the absence of ligand. Experiments in which Na^+-loaded vesicles were diluted into K^+ and vice versa showed that the ion flux was not specific with regard to these two cations. Ca^{2+} was poorly transported relative to monovalent cations. To demonstrate that these conductance changes were not caused by simple binding to the vesicles of monoclonal antibodies, the same series of monoclonal antibodies was tested in the membrane vesicle system. These reagents had no effect on the uptake of TPP^+, demonstrating the specificity of the permeability changes observed.

The results of TPP^+ uptake triggered by immune complexes or 2.4G2 suggest a role for the $Fc_{\gamma 2b/\gamma 1}R$ in the conductance changes, but did not rule out the possibility that the channel is formed by interaction of the receptor with another plasma membrane protein(s). To address this possibility, the purified FcR was reconstituted into phospholipid vesicles by detergent dialysis from octylglucoside, and the uptake of TPP^+ into the vesicles was measured in the presence or absence of 2.4G2 IgG (Young *et al.*, 1983*b*). Relative to the control, a substantial amount of TPP^+ was taken up in the presence of 2.4G2 IgG, demonstrating that the conductance change seen in the plasma membrane vesicles and intact J774 cells is attributable to the presence of the FcR, and not to other plasma membrane proteins.

The conductance changes observed could be caused by a nonspecific change in monovalent cation permeability rather than the formation of ion channels. To study permeability changes on a microscopic rather than a macroscopic level, as in the TPP^+ uptake experiments. $Fc_{\gamma 2b/\gamma 1}R$ was reconstituted into planar bilayers by the method of Montal and Muller (1972), and the ionic current flowing through the membrane was measured after the addition of appropriate ligands. The receptor was reconstituted in a lipid monolayer on one side of a two-cham-

bered apparatus by rapid dilution of a solution containing phospholipid, octylglucoside, and purified $Fc_{\gamma 2b/\gamma 1}R$. The other chamber did not contain FcR. When the level of the buffer in both chambers was raised to span the annulus separating the two chambers, an asymmetric bilayer was thus formed. Addition of 2.4G2 IgG, or immune complexes, but not normal rabbit IgG to the *cis* chamber, in which the FcR was initially reconstituted, resulted in a large increase in membrane conductane when a potential was imposed between the two compartments. This conductance increase decayed with time, and addition of more ligand to the *trans* chamber had no effect on conductance. When the amount of FcR used for the reconstruction was sufficiently diluted, and the salt concentration was raised to 1 *M* to increase the amount of current, off-on conductance jumps were observed compatible with single channels opening and closing. The conductance of these events was 60 ± 5 pS (pico Siemens unit) and the current-voltage plot for single channels showed a linear relationship.

One of the powerful applications of immunologic reagents is to perturb the normal working of biologic systems in order to dissect the functional significance of various epitopes that the antibodies may recognize. 2.4G2 IgG has been shown, not surprisingly, to interfere with ADCC mediated by macrophages elicited with bacillus Calmette-Guérin (BCG) (Nathan *et al.*, 1980). Perhaps of more interest is the study of West Nile virus, which has been advanced as a model for Dengue hemorrhagic fever. The infectivity of West Nile virus, a flavivirus that can replicate in cells of the $P388D_1$ mouse macrophage cell line, is increased 100-fold by subneutralizing amounts of IgG. This increase in infectivity is attributable to the Fc domain of the IgG and is reversed almost totally by the addition of 2.4G2 IgG (Peiris *et al.*, 1981); 2.4G2 IgG has also been reported to act as a B-cell mitogen and to stimulate a polyclonal antibody response (Lamers *et al.*, 1982). We have, however, been unable to confirm these observations (E. Pure and J. Unkeless, unpublished results).

Another area in which monoclonal antibodies have provided useful probes is in the study of induction and regulation. Hamburg *et al.* (1980) demonstrated that, although there was enhancement of macrophage phagocytosis of IgG-opsonized E after treatment with type I interferon, there was no increase in the amount of 2.4G2 bound to the induced cells. Echoing these results, Ezekowitz *et al.* (1983) report that IgG2a binding, but not IgG2b binding, is selectively enhanced after stimulation of mouse macrophages by BCG. Yoshie *et al.* (1982) found evidence for increased levels of both $Fc_{\gamma 2a}R$ and $Fc_{\gamma 2b/\gamma 1}R$ after induction with α- and β-interferon of the mouse macrophage cell line RAW 309 Cr.1.

It is not clear whether the different mouse Fc receptors have specialized physiologic functions. Ralph *et al.* (1980) found that all IgG subclasses in mouse mediate phagocytosis and lysis of IgG-coated E. However, Ezekowitz *et al.* 1983) found that IgG2a complexes stimulate the oxidative burst of BCG-activated macrophages more efficiently. Further suggestion that $Fc_{\gamma 2a}R$ may be of

particular interest in tumoricidal/microbicidal activity comes from Matthews *et al.* (1981), who found that macrophage cytotoxicity and *in vivo* protection against the 775 murine adenocarcinoma cell line were mediated by mouse IgG2a antibody. Supporting the hypothesis that different receptors have different functions, Nitta and Suzuki (1982) found differences in cyclic nucleotide responses after adherence of IgG2a- and IgG2b-sensitized E. Clearly further work is needed in this area.

IX. HUMAN Fc RECEPTOR

The analysis of human Fc receptors has largely focused on binding to monocytes of different subclasses of human IgG and has resulted in a rank order of cytophilicity in which IgG1 = IgG3 > IgG4 ≫ IgG2 (reviewed in Dickler, 1976; Unkeless *et al.*, 1981). Competitive binding experiments between different human IgG subclasses for binding to the U937 monocytic cell line failed to reveal any heterogeneity in Fc binding sites (Anderson and Abraham, 1980). However, Messner and Jelinek (1970), and Huber *et al.* (1969) reported lack of binding of some anti-Rh_0 sera to neutrophils, suggesting a possible difference between neutrophil and monocyte receptors. These results were recently confirmed by Kurlander and Batker (1982), who demonstrated that human IgG1 oligomers bound with 100- to 1000-fold higher avidity to monocytes than to neutrophils. The neutrophil receptor is thus a relatively low-avidity receptor ($Fc_\gamma R_{10}$) that is probably triggered only by immune complexes, as compared with the receptor on monocytes ($Fc_\gamma R_{hi}$), which binds IgG1 monomer with a $K_a > 10^8\ M^{-1}$.

Analysis of human Fc receptors using monoclonal antibodies has provided solid evidence for human Fc receptor heterogeneity. Fleit *et al.* (1982) have isolated $Fc_\gamma R_{10}$ monoclonal antibody, 3G8, by screening hybridoma supernatants for inhibition of neutrophil rosetting with IgG-sensititized erythrocytes. The Fab fragment of 3G8 retained its potent inhibitory capacity against neutrophil Fc receptor. Immunoprecipitation of labeled neutrophils by 3G8 Fab-Sepharose revealed two poorly resolved peptides, of 53,000 and 66,000 M_r, which resemble the Fc receptor from mouse macrophages immunoprecipitated with 2.4G2 Fab-Sepharose. A monoclonal antibody, B73.1, with comparable sepcificity against $Fc_\gamma R_{10}$ has been isolated by Perussia *et al.* (1983). B73.1 IgG stains human NK cells brightly, and, like 3G8, does not react with blood monocytes, V937, or HL-60 cell lines. Affinity chromatography on IgG-Sepharose of ^{125}I-surface-labeled detergent lysates from human mononuclear cells and neutrophils resulted in a broadly migrating protein of 52,000–64,000 M_r (Kulczycki *et al.*, 1981). Comparable experiments using lysates of the U937

cell line resulted in molecules of 72,000 and 40,000-43,000 M_r (Anderson, 1982).

Although there appears to be structural similarity between the human Fc receptor recognized by 3G8 and the mouse $Fc_{\gamma 2b/\gamma 1}R$, the cellular distribution of the two antigens is very different. The 3G8 antigen is present on all neutrophils and eosinophils, on 15% of peripheral blood B cells, and on 6% of E-rosetting cells. However, the 3G8 antigen is absent from peripheral blood monocytes and from the promyelocytic HL-60 and monocytic U937 human cell lines, which have high-avidity receptors for human IgG1 (Anderson and Abraham, 1980; Crabtree, 1980). Although absent from monocytes, 60% of macrophages isolated from resected lung tissue bear the 3G8 determinant, as determined by immunofluorescence staining. This result, plus the observation that the antigen appears on monocytes cultered *in vitro* for 7 days, suggests that the low-avidity Fc receptor for IgG ($Fc_\gamma R_{10}$) is either an inducible protein or a marker of a particular stage in the monocyte-macrophage differentiative pathway.

We have studied the induction of $Fc_\gamma R_{10}$ on HL-60 cells after treatment with retinoic acid or dimethyl sulfoxide (DMSO) and on chronic myelogenous leukemia (CML) cells as models for the expression of $Fc_\gamma R_{10}$ during differentiation. Immunofluorescent staining of bone marrow cells for $Fc_\gamma R_{10}$ and counterstaining for nuclear morphology with *p*-phenylenediamine, a free radical scavenger used to block fluorescence bleaching (Johnson and Nogueira Araujo, 1981), revealed staining on cells at the metamyelocyte or later stages, but not on less differentiated forms. In agreement with these observations, the uninduced HL-60 cell line, which has the morphologic appearance of cells at the promyelocyte stage of differentiation, did not express the 3G8 antigen. After induction with DMSO or retinoic acid, however, both of which have been shown to drive HL-60 to more mature myeloid stages (Collins *et al.*, 1978), 5-40% of the cells synthesize $Fc_\gamma R_{10}$ (Fleit *et al.*, 1984). Finally, although the more mature cells in the peripheral circulation of patients with chronic myelogenous leukemia (CML) have the same number of 3G8 binding sites and bind the same amount of IgG in immune complexes as do peripheral neturophils, immature cells from CML patients are completely negative for 3G8 antigen.

X. OTHER MOUSE MACROPHAGE ANTIGENS AND STUDIES ON MEMBRANE RECYCLING

Immunizations of rats with mouse macrophage cell lines have resulted in the generation of monoclonals directed against antigens which, although not unique to macrophages, have been extremely useful in the analysis of membrane flow and recycling of membrane proteins. Monoclonal antibodies used in this way

include 2D2C, which immunoprecipitates a glycoprotein of 90,000 M_r and recognizes an alloantigen present on DBA/2, Balb/c, and CBA, but not A, B10, B10.D2, or AKR mice (Nussenzweig *et al.*, 1981); 1.21J, which recognizes Mac-1, thought to be the CR_3 receptor (Beller *et al.*, 1982); 2E2A, which recognizes a protein of 82,000 M_r; F4/80, which immunoprecipitates a macrophage-specific glycoprotein of 150,000 M_r (Austyn and Gordon, 1981); 2F44, which recognizes a protein of 42,000 M_r; 25-1, which recognizes H-2D^d; and 2.6, which immunoprecipitates a protein of 20,000 M_r (Mellman *et al.*, 1980). These proteins together constitute about 25% of the total plasma membrane protein subject to iodination by lactoperoxidase and glucose oxidase.

The relative distribution of these proteins in the plasma membrane was compared with the distribution in vesicles, which were labeled after pinocytosis of lactoperoxidase (Mellman *et al.*, 1980). In most cases, the relative distribution of proteins labeled on the plasma membrane was the same as the distribution of proteins in the labeled vesicles, arguing against exclusion of these proteins in pinocytic vesicles. However, one protein, recognized by monoclonal antibody 2.6, was preferentially represented in the labeled pinosome proteins relative to the plasma membrane (Mellman *et al.*, 1980). Muller *et al.* (1983) have examined the protein composition of endocytic vacuoles formed by macrophage phagocytosis of Latex particles and find the plasma membrane proteins are present in the same relative amounts in phagosomes, with the notable exception of the antigen precipitated by 2.6, which was present at 7-fold the level found on the plasma membrane. The 2.6 antigen was present on macrophages, absent from lymphocytes, and present in large amounts on dendritic cells, platelets, and granulocytes (Nussenzweig *et al.*, 1981). The function of the molecule is unknown, but it is tempting to speculate that it is involved in the specialized phagocytic and/or secretory functions carried out by these cell types.

Patients with circulating immune complexes often have a defect in the rate of clearance of IgG-coated erythrocytes (Frank *et al.*, 1983). This defect may be secondary to internalization and clearance of Fc receptors from the surface of the phagocytic cells. Using rabbit antisera specific for the mouse $Fc_{\gamma 2b/\gamma 1}R$, prepared by immunization with protein purified by affinity chromatography on 2.4G2 Sepharose, Mellman *et al.* (1983) studied the rate of degradation of $Fc_{\gamma 2b/\gamma 1}R$ after phagocytosis of erythorocyte ghosts coated with IgG and found a significantly increased rate of degradation ($t_{1/2}$: <2 hr) relative to the free receptor of ($t_{1/2}$: 10 hr). The rates of turnover of other membrane proteins examined were not affected by phagocytosis of the opsonized ghosts. After ingestion of the opsonized erythrocyte ghosts, there was a small (10%) transient decrease in the binding to the plasma membrane of monoclonal antibodies 2D2C, 1.21J, and an antibody specific for H-2D^d, but a large (>60%) decrease in 2.4G2 (anti-$Fc_{\gamma 2b/\gamma 1}R$) binding, which remained depressed over the next 24 hr. Thus, ligand can profoundly affect the subsequent turnover and the degradation

of $Fc_{\gamma 2b/\gamma 1}R$ while not altering the turnover and recycling of other plasma membrane proteins.

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