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Chapter 118
Macrophages

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Mouse and human monocyte or macrophage antigens defined by monoclonal antibodies

A large number of anti-mouse and anti-human macrophage or monocyte monoclonal antibodies (MAb) have been recently obtained and are proving to be invaluable reagents of extraordinary specificity for the study of macrophage differentiation, function, and surface antigen structure. Information on these antibodies is summarized in Tables 118.1 and 118.2, which are meant to be self-explanatory.

In the mouse, at least five antigens distinguishable by relative molecular mass have been found, which are present on macrophages but not on lymphocytes (Table 118.1). Two physiologically important receptors have been defined. The complement receptor type 3 (CR_3) is identical to Mac-1 [6]. The CR_3 binds a cleaved and inactivated form of C3b, namely C3bi. The mouse Fc receptor for IgG1 and IgG2b is defined by the 2.4G2 MAb, and is present on macrophages and some lymphocytes. Further antigens have not been defined biochemically, but appear to have distinct distributions on functional subpopulations.

Through the vigorous efforts of many different laboratories, more than forty monoclonal antibodies to human monocyte or macrophage antigens have been obtained (Table 118.2). Most of the antibodies have not been cross-compared, and in only a few cases have the antigens been structurally characterized. Many of the antigens have similar cell distributions. Therefore the actual number of different antigens may be considerably less than forty. The antigens are grouped according to cell distribution. The first group is present on monocytes and granulocytes. It includes the OKM1, Mo1, and human Mac-1 antigens, which appear identical to one another and to the human complement receptor type 3 (CR_3). The second group of antigens is only weakly present on granulocytes, and the third group of antigens is present only on monocytes or macrophages. The fourth group of antigens shows distribution on other cell types. Several antigens which are present on platelets as well as monocytes have been shown to be bona fide monocyte membrane proteins, rather than platelet antigens. Adherence of platelets to monocytes occurs in some monocyte isolation protocols [57]. The Fc receptor of neutrophils, which is also present on macrophages, and the complement receptor type 1 (CR_1), which binds C3b, have been identified with function-blocking MAb. These and additional anti-human myeloid MAb have recently been characterized in the Strand International Leucocyte Workshop [176].

The remainder of this chapter illustrates the use of monoclonal antibodies in the study of macrophage differentiation, the structure of macrophage surface antigens, the interrelationship with other types of surface structures as detected by cross-reaction, and the use of MAb as probes in the study of macrophage surface receptor structure and function. Most of the examples are taken from the work of the authors and their collaborators.

Mac-1 antigen

Mouse Mac-1 was the first antigen to be defined by MAb that is present on myeloid cells and not on lymphocytes [1]. More recently, several other MAb to it have been obtained [2,58,59]. The Mac-1 antigen contains two subunits, an α subunit of Mr 170 000 (relative molecular mass) and a β subunit of Mr 95 000. The α and β subunits are not linked by disulphide bonds, but are tightly non-covalently associated in an $\alpha_1\beta_1$ complex [60,61]. Antibodies specific for the Mac-1 antigen bind to the α subunit [59]. The LFA-1 antigen on lymphocytes has a different α subunit associated with the same β subunit (see

Table 118.1. Murine macrophage antigens defined by monoclonal antibodies

Antibody/antigen designation	Antigen polypeptide Mr $\times 10^{-3}$	Cellular distribution	Distinguishing features; functional features	References
Mac-1	95 170	Resident and exudative peritoneal macrophages, splenic histiocytes, granulocytes, PB monocytes, NK killers	Blocks type 3 complement receptor (CR); absent from Langerhans' cells, interdigitating, and lymphoid dendritic cells; Ml/70 MAb binds to human PB monocytes, PMN, NK killers; 170K chain bears epitope	[1–6]
Mac-2	32	TG-peritoneal exudate macrophages; weak or absent from resident and *Listeria* exudative macrophages	Present on Langerhans' and interdigitating, but not follicular dendritic cells; on epithelial cells; absent from granulocytes	[7–10]
Mac-3	110	Macrophages, granulocytes	On Langerhans' and interdigitating, but not follicular dendritic cells; on epithelial and endothelial cells	[7,9–11]
54-2	180	Cultured bone marrow macrophages, TG-peritoneal exudate macrophages, mast cells	Absent from PB monocytes and resident macrophages	[12,13]
F4/80	160	PB monocytes, resident and induced macrophages	On 8% bone marrow cells and on P815; macrophage activation reduces expression; 75K trypsin fragment bears epitope	[14–16]
2.4G2	47–70	Macrophages, PMN, B cells, J774, FcR bearing T cells	Blocks binding of IgG1 and IgG2b Fc to FcRII; protease-resistant	[17,18]
ACM.1		Peritoneal macrophages activated by pyran or *C. parvum*; absent from resident and protease exudative peritoneal macrophages	Cytotoxic for tumoricidal macrophage effectors	[19]
M43, M57, M102, M143		Bone marrow macrophages, resident and exudative peritoneal macrophages	Define different cytotoxic macrophage subpopulations	[20]

below), 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 [61]. 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 [Simon & Springer, unpublished]. 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 Mr 161 000 and 87 000, respectively [62]. Assembly into the $\alpha_1\beta_1$ complex appears to precede processing of the subunits to their mature relative molecular mass, which presumably involves changes in glycosylation.

Mac-1 appears to be a universal macrophage marker [8]. It is expressed on >95% of peritoneal resident macrophages and macrophages elicited by thioglycollate, lipopolysaccharide, peptone, *Listeria monocytogenes*, and concanavalin A. The average amount of Mac-1 expressed per cell varies by no more than twofold among these different populations. Correlating with their larger size, thioglycollate-elicited macrophages express the highest number of anti-Mac-1 MAb binding sites per cell, namely 1.6×10^5. Mac-1 is present on splenic macrophages in the red pulp and in the marginal zones surrounding the

Table 120.2. Human monocyte or macrophage antigens defined by monoclonal Ab

	Antigen, antibody designation	Ab subclass	Antigen polypeptide Mr × 10^{-3}	Monocytes	Macrophages	Granulocytes	NK/LGL Cells	Lymphocytes	Activated lymphocytes	Platelets	% Bone marrow	Mon. prec.	Gran. prec.	CFU-GM	Eryth. prec.	Megakaryocytes	Other characteristics	ATCC[a]	References
I	OKM1,9 and 10	γ2b,γ1,γ2	155,94	+		+	+	−	−	−							CR_3[b]		[21–26]
	Mac-1, M1/70	γ2b		+	+	+	+	−	−		40	+	+	−	−		CR_3	+	[3,6,27]
	Mol	μ,γ2b	155,94	+	+	+	+	−	−	−	21	+	+	−	−	−	CR_3		[28,25,29]
	B2.12			+		+		−		−									[30]
	M522			+		+	+	−											[31]
	MMA	μ		+		+			+	−	25			+			Accessory cells[c]	+	[32]
	S5–25	μ		+		+							+						[33]
	S4–7	μ	145	+		+		−					+		−				[33]
	My7	γ1	160	±		+		−		−	5			+	−	−			[34,35]
	My8	γ2b		+		+		−		−	32			−	−	−			[34,35]
	B13.4, B48.4	μ		+		+		−		−			+		−				[36]
	20.2	γ2b		+	+	+	−	−	−	−	14	+	−		−				[37]
II	B43.4	γ2b		+		+		±		−			+		−				[36]
	B34.3	γ1		+		+		−		−			±		−				[36]
	AML-2-23	γ1		+	+	+		−		−									[38]
	MØP-9	γ2b		+	+	±		−	−	−									[39]
	MØS-1	γ2a		+	+	±		−	−	−									[39]
	MØS-39	γ2a		+	+	±		−	−	−									[39]
	63D3	γ1	200	+	−	±		−									Accessory cells[c]	+	[40–42]
	Mo6	γ1	80	+		±						+	+						[29]
	My4	γ2a		+		±		−		−	5			−	−	−			[34,35]
III	Mac-120		120?	+		−		−									Accessory cells[d]		[43]
	Mo2	μ	55	+	+	−	−	−		−							Accessory cells[e]		[25,44–45]
	Mo3	μ		+	−	−	−	−		−									[44]
	61D3	γ1	23,55	+		−		−											[40]
	B44.1	μ		+		−		−		−			+		−				[36]
	D5D6	μ		+		−		−	−					+					[46]
	C10H5	μ		+		−		−	−					±					[46]
	MØP-15	γ1		+	+	−		−	−	−									[39]
	MØP-7	γ1		±	+	−		−	±	−									[39]
	MØR-17			±	+	−		−	±	−									[39]
	My3	μ		+		−		−		−	4			−	−	−			[34]
	PHM2	γ1		+	+	−		−											[47]
	PHM3	γ2a		+	+	−		−											[47]
	ID5			+	+	−		−											[41]
IV	UC45	μ	60	+													Fibrin[g]		[49,50]
	Mo4	μ		+	+	±	−	−		+									[44]
	OKM3 and 6	μ,γ1	116	+		−	−	−		+									[26]
	OKM5 and 8	γ1	88	+		−	−	−		+							Accessory cells[f]		[26]
	MPA,10-75-3	γ2a	135,93	+		−		−		+						+			[51]
	5FI	μ		+		−		−		+	8	+	−		+				[52]
	20.3	μ		+	+	−	−	−	−	+	10	+	+	+	+	+			[37]
	4F2	γ2a	80,40	+				±	+									+	[53]
	S5–7	γ1	20	+		±		T					+		+				[33]
	$Fc_\gamma R_{lo}$, 3G8	γ1	66,53	−	+	+		±									$Fc_\gamma R_{lo}$	+	[54]
	CR_1	γ1	205	+	+	+		B									CR_1[i]		[55,56]

a Deposited with the American Type Culture Collection, 12301 Parklawn Drive, Rockville, MD 20852-1776.
b Complement receptor type 3, specific for C3bi.
c T cell proliferative responses to mitogens.
d T cell proliferative responses to mitogens and antigens.
e Primary Ig responses.
f Induction of autologous mixed lymphocyte reaction.
g Fibrin polymerized on processes of monocytes and neurons.
h Fc receptor on neutrophils, distinct from that on monocytes.
i Complement receptor type 1, specific for C3b; is also on erythrocytes.

periarteriolar lymphoid sheath [8], and on lymph node medullary cord macrophages [9]. 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 [9].

The Mac-1 antigen is also present on exudate granulocytes and granulocytic precursors in the bone marrow [1], and on natural killer cells. Immunofluorescent cell sorter experiments have shown that cells with natural killing activity obtained from the nylon wool non-adherent fraction of peritoneal exudates are Mac-1$^+$ [5]. 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 [3]. Human Mac-1 is equivalent to the OKM1 and Mo1 antigens [6]. The human granulocyte-monocyte precursor cell (CFUgm) appears to be Mac-1$^-$ [27], Mo1$^-$ [35]. Thus, granulocytes and monocytes or macrophages become Mac-1$^+$ after divergence from their common stem cell. NK cells are renewed from the bone marrow [63], 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 lack antigens characteristic of T cells [3,22,64,65], while alloactivated or 'cultured' NK cells express some T cell markers and may be either Mac1/OKM1 positive [66] or negative [67–69].

Mac-1 distribution on tumour cells in the monocyte or macrophage lineage parallels that on normal cells [70]. The immature myelomonocytic leukaemia 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 haematopoietic 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 [6]. The M1/70 anti-Mac-1 MAb strongly inhibits complement receptor-mediated rosetting of E-IgM-C (erythrocyte–IgM antibody–complement complexes). Lack of inhibition by a panel of eight other antibodies, including anti-Mac-2, anti-Mac-3, and anti-H-2 and anti-pan-leucocyte MAb which bind to a similar number of sites per cell as anti-Mac-1, demonstrates the 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 [71]. 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 [6]. 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 the above 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 [23]. *S. 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 to that reported for the CR_3 (reviewed in refs. 71 and 72). The CR_3 is present on monocytes and neutrophils [73] as is Mac-1. It also is present on 6–10% of human blood lymphocytes [73,74], in agreement with the finding of Mac-1 on the 'null' subpopulation of $\sim$10% of human lymphocytes which contain NK activity [3]. There are conflicting findings on the presence of the CR_3 on glomerular epithelial cells [6,75]; kidney is negative for Mac-1 as shown 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 [73]. 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 due to 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 [76].

The Mac-1 and LFA-1 family

Mac-1 is one member of a family of structurally related leucocyte surface antigens. Another member, LFA-1, has been discovered that is distinct from Mac-1 in cell distribution, function, and α subunit structure, but appears to utilize 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 [58]. Some of these MAb defined the LFA-1 antigen, which contains two polypeptide chains of Mr 180 000 and 95 000. MAb to LFA-1 block killing by inhibiting formation of the adhesion between the CTL and the target cell [58,77]. 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 [58]. LFA-1 is present on B lymphocytes and myeloid cells as well as T lymphocytes [78], 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 [58], and adhesion of macrophages to C3bi-coated cells, which is mediated by the CR_3, are Mg^{2+}-dependent [79,80].

The Mac-1 and LFA-1 β subunits of Mr 95 000 are identical by peptide mapping and by complete immunologic cross-reactivity [59,60]. Similar peptide map results were obtained for Mac-1 and an antigen probably identical to LFA-1 [81]. MAb cross-reactive for Mac-1 and LFA-1 have been shown to bind to an epitope on their β subunits [59]. MAb that are specific for Mac-1 or LFA-1 bind to α chain epitopes. The α subunits are not cross-reactive, as shown with both monoclonal and conventional antisera, and they have different tryptic peptide maps. However, sequencing of their N-termini has shown 40% amino acid sequence homology [Teplow, Dreyer & Springer, unpublished], 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 [59]. 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 [24]. Human Mac-1 is homologous to mouse Mac-1 as shown by monoclonal antibody cross-reaction, identical cell distribution of the antigens [3], and identical association with the CR_3 [6]. 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 [23,24,29]. Human LFA-1 is equivalent to mouse LFA-1 and shares a common β subunit with OKM1 [24]. Furthermore, yet a third antigen with a distinct α chain of Mr 150 000 has been found to be associated with the same β subunit. It is found on granulocytes and monocytes [24]. Thus an interrelated family of three different cell surface molecules has been described; they utilize 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.

A disease has been discovered in which the Mac-1/OKM1 molecule is deficient [157,158]. Patients suffer recurring bacterial infections and may die in early childhood. The LFA-1 and p150,195 molecules are also lacking in these patients [158,159], but other surface receptors such as the CR1 and FcR are normal. Thus, the family of molecules sharing the 95 000 Mr β subunit is specifically deficient. Biosynthetic studies show that an intracellular LFA-1 α chain precursor is synthesized in normal amounts, but does not reach the cell surface because no β chain is present [159]. Thus, the primary defect in the disease appears to be in the β subunit, and association of α and β is required for normal processing and surface expression. Inheritance of the defect is autosomal recessive. Granulocytes from affected individuals are deficient in the C3bi receptor, in aggregation, and in adherence to surfaces [157–159].

Mac-2 antigen

Mac-2 is a macrophage surface antigen of Mr 32 000. It is biosynthesized by macrophages [8], and the precursor identified by 5 min pulse labelling has the same relative molecular mass as the mature antigen [Ho & Springer, unpublished]. Isoelectric focussing shows that Mac-2 is a basic polypeptide having a pI in the range of 7–8. It focusses in a position very close to that of the invariant chain of Ia, which is of Mr 31 000 [82]. However, there are no identities between the methionyl tryptic peptides of Mac-2 and the Ia invariant chain [Ho & Springer, unpublished]. 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 [8]. Among resident macrophages and five different types of elicited peritoneal macrophages that were studied, only thioglycollate-elicited macrophages showed strong expression by immunofluorescent flow cytometry and immunoprecipitation of ^{35}S-methionine labelled antigen. Mac-2 is expressed equally strongly by macrophages one day and four days after elicitation with thioglycollate. Thus cells recruited into the peritoneum after one day are already committed to

the synthesis of Mac-2. Biosynthesis of Mac-2 by resident peritoneal macrophages and macrophages elicited by peptone, lipopolysaccharide, concanavalin 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 [9]. Mac-2 has been found on all mature macrophage cell lines which have been examined, and is absent from lymphoid and primitive erythroid and myelomonocytic lines. However, in contrast to Mac-1 and Mac-3, Mac-2 is not expressed by the M1 cell line after induction of maturation [70].

Resident peritoneal macrophages, or those elicited by a variety of agents, synthesize and express on their surface similar amounts of the Mac-1 antigen [8]. Mac-1 is thus a constitutive macrophage marker, whereas Mac-2 and Ia [83] appear to be inducible surface components. The induction of Mac-2 and Ia is controlled independently, since some macrophages, such as thioglycollate-elicited ones, 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 [9]. 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 strain 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 [84].

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 relative molecular mass [85]. 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.

Mac-3 antigen

Mac-3 [62] is a less abundant antigen, present in 3.6×10^4 cell surface sites per thioglycollate-elicited macrophage and also in the cell interior. It is expressed in similar quantities on resident peritoneal macrophages and on macrophages elicited by a variety of agents. It is on macrophages in a number of tissues examined by immunoperoxidase staining of thin sections [9,86] and it is on all of eight different macrophage cell lines [70]. Mac-3 is a glycoprotein and appears as a somewhat diffuse band in SDS-PAGE. Mac-3 is found on the surface of macrophages and granulocytes but on no other haematopoietic cell types. It is also found on some non-haematopoietic cell types, giving highly specific intracellular, granular staining patterns on epithelial and endothelial cells in a variety of tissues [9]. Both liver parenchymal and Kuppfer cells are stained, and staining of bile canaliculi is particularly intense. Intestinal epithelial cells are stained only on their luminal border.

Recently, Mac-3 has been shown to be a lysosome-associated membrane protein [177]. Expression on the cell surface appears confined to myeloid cells.

An unusual feature of Mac-3 is that its relative molecular mass varies from 100 000 to 170 000, depending on the type of elicited macrophage or macrophage cell line from which it is isolated [70]. Macrophages elicited by different agents synthesize identical Mac-3 precursors of Mr 74 000 which are processed in 15 min to the higher Mr mature forms which vary in Mr depending on the type of macrophage population [62]. This shift appears to be due to glycosylation. Recent studies of Mac-3 carbohydrate show that both high mannose, complex, and lactosaminoglycan moieties are present [Mercurio, Robbins & Springer, unpublished]. 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.

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 [87], 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*

Table 118.3. Properties of murine dendritic cells and macrophages[a,b]

Property/ antigen	Macrophage	Langerhans' cell	Interdigitating dendritic cell	Follicular dendritic cell	Lymphoid dendritic cell
Ia	+/−[94]	+[95]	+[95]	−[92]	+[96,97]
Mac-1	+[1]	−[9,10]	−[9,10]	−[9,10]	−[97]
Mac-2	+[8]	+[9,10]	+[9,10]	−[9,10]	ND
Mac-3	+[11]	+[9,10]	+[9,10]	−[9,10]	ND
Ly-5/CLA	+[1,98]	+[9,10]	ND	ND	+[97]
FcR	+[99]	+[9,10,100]	ND	+[92]	−[97,101]
C3b R	+[72]	+[102,c]	ND	+?[92,d]	−/+[101,d,e]

a The terminology is that of Tew *et al.* [91]
b Modified from Haines *et al.* [10].
c Langerhans' cells bear the receptor for C3b, and lack receptors of C3bi and C3d [105].
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 $C3R^+$ [103].

[88,89]. 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 [90,91]. This anatomical association, and the morphological resemblance to Langerhans' cells, including the sharing of the unique, tennis racket-shaped Birbeck granule, suggests 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 [90,92]. The lymphoid dendritic cell of Steinman & Cohn [93] has been isolated in suspension, has a dendritic morphology, and is an active accessory cell for lymphocyte responses. Its exact 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 [89].

The surface markers of dendritic cells and macrophages have been compared (Table 118.3). All except follicular dendritic cells bear Ia antigen, which is important in induction of antigen-specific T lymphocyte responses [104]. Macrophages can be distinguished from all of 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 [105]. The lymphoid dendritic cell of Steinman & Cohn also lacks Mac-1. Interestingly, Langerhans' cells and interdigitating cells express the Mac-2 and Mac-3 antigens, whereas follicular dendritic cells are negative for both antigens. The common $Mac\text{-}1^-2^+3^+$ phenotype of Langerhans' cells and interdigitating dendritic cells supports the ideas, based on morphologic similarities, that these may be ontogenetically and functionally related cells localized in different anatomic sites, and that these cells differ from follicular dendritic cells. It will be interesting to learn whether lymphoid dendritic cells are also $Mac\text{-}2^+3^+$. All cell types tested, i.e. macrophages, Langerhans' cells, and lymphoid dendritic cells, share the Ly 5 or common leucocyte antigen (CLA) molecule. This marker has thus far only been found on haematopoietic cells [78,98,106,107].

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 [70] and humans [108] with the M1 and U937 tumour line models, respectively. Studies in humans have shown that the granulocyte-monocyte colony forming unit is $Mac\text{-}1^-$ or low in Mac-1 [27,35]. Mac-1 expression is increased during differentiation of peripheral blood monocytes to peritoneal macrophages [1]. 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. Co-expression 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 anatomical 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.

There are few surface markers which are specific for what haematologists 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 haematologists or immunologists. The cells of the immune defence 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-vs.-host reactions [109], and endothelial cells become Ia^+ in response to γ-interferon secreted by lymphocytes [110].

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 was found to be present on elicited but not resident macrophages [12], and also to mark mast cells [13]. Mac-2 and Mac-3 are present on macrophages and not lymphocytes, but are also present on epithelial, and for Mac-3, additional non-haematopoietic cells. The OKT6 antigen, which was originally thought to be thymocyte specific, has recently also been found on Langerhans' cells [111]. The Thy-1 antigen has long been used as a marker for distinguishing T lymphocytes from other cells of the haematopoietic 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 [112,113]. 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 natural killer/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 [114]. On resident macrophages, the CR_3 is synergistic with the FcR for phagocytosis [115]. What is the role of the CR_3 (Mac-1) on NK and ADCC cells, which are non-phagocytic? When target cells are coated with C3bi in addition to IgG, lysis by ADCC effectors is greatly enhanced [74]. This suggests that the CR_3 synergizes with the FcR in the killing reaction. Whether the presence of C3bi on target cells would enhance natural killing has not yet been tested.

Mouse Fc $_{\gamma 2b/\gamma 1}$R

The analysis of mouse Fc receptors (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 [116,117], while that binding IgG2b and IgG1 ($Fc_{\gamma 2b/\gamma 1}R$), and IgG3 [118] are resistant to trypsin. Although the results of competition experiments with monomeric IgG myeloma proteins are equivocal with respect to FcR heterogeneity [119,120], competition experiments using aggregated IgG and immune complexes of different subclasses indicate that there are three different FcR on macrophages [117,118,121]. Comparable results have been obtained in rats [122]. 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 $Fc_{\gamma 3}R$ receptor, respectively [17,118].

The mouse macrophage FcR specific for IgG2b and IgG1 immune complexes has been characterized using a rat monoclonal antibody, 2.4G2 [17,18]. The monoclonal antibody was isolated following 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, following pre-incubation 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 leucocytes (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 [97].

The $Fc_{\gamma 2b/\gamma 1}R$ was purified by affinity chromatography on 2.4G2 Fab-Sepharose 4B [18]. Non-ionic detergent lysates of J774 tumours or cultured J774 cells were adsorbed on an affinity column, which was then washed with Nonidet P-40/sodium dodecyl sulphate (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 [123]. 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 Mr 47 000 and 60 000. The peptides are glycosylated, and can be labelled by galactose oxidase oxidation followed by reduction with NaB_3H_4. In two-dimensional isoelectric focussing-SDS-PAGE, the $Fc_{\gamma 2b/\gamma 1}R$ exhibits the typical decrease in Mr from acidic to basic species. The isoelectric point of the purified receptor is broad, with a pI from 4.7 to 5.8. This was later confirmed by Lane & Cooper [124], who isolated FcR by affinity chromatography using both 2.4G2 IgG and affinity chromatography on IgG2b-Sepharose. Lane & Cooper [124] also observed small differences in Mr and isoelectric point between Fc-binding proteins isolated from IgG2a-Sepharose compared to proteins eluted from IgG2b-Sepharose, suggesting that the two receptors have structural differences. Others have isolated similar Fc-binding proteins [125–127] from mouse macrophages or macrophage cell lines. However, differences between proteins isolated on IgG2a vs. 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 Mr peptide and a concomitant increase in the amount of the lower Mr 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 tryptic and chymotryptic maps of the two peptides is consistent with that interpretation (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 Mr was observed, with the largest species from the B cell line WEHI-213, 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, although not to IgG3-coated beads. However, in the presence of detergent, purified and labelled $Fc_{\gamma 2b/\gamma 1}R$ bound best to IgG2b aggregates, less well to IgG1 aggregates, and did not bind to either IgG2a or rabbit IgG [unpublished data]. The purified FcR in the absence of detergent formed aggregates of large size (S value = 15). The authors attribute the previously observed lack of specificity to the magnification of a low avidity of binding due to 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 defence mechanisms, which range from phagocytosis of the offending particle to release of hydrolytic enzymes, superoxide, prostaglandins, and leucotrienes. 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 [128]. The effect of exposure of J774 macrophage-like cells to immune complexes and the anti-$Fc_{\gamma 2b/\gamma 1}R$ monoclonal 2.4G2, as well as other monoclonal antibodies, was tested [129]. 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 which lasted from 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 by the depolarization in response to immune complexes. Other monoclonal antibodies—2D2C, 2E2A, and 1.21J—which all recognize major antigenic determinants on J774 cells [2,97,130] 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 exam-

ined by TPP^+ uptake following dilution of vesicles with entrapped cations into isotonic sucrose containing ligands and labelled TPP^+ [131]. 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 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 due to simple binding to the vesicles of monoclonal antibodies, the same series of monoclonal antibodies discussed above 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 (or proteins). 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 [131]. 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 due to the presence of the FcR, and not to other plasma membrane proteins.

The conductance changes observed could be the result of a non-specific 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 [132] 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-chambered 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 conductance 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 reconstitution was sufficiently diluted, and the salt concentration was raised to 1 M to increase the amount of current, conductance jumps off and on were observed which are compatible with single channels opening and closing. The conductance of these events was 60 ± 5 pS and the current–voltage plot for single channels showed a linear relationship [160].

One of the powerful applications of immunologic reagents is to perturb the normal working of biological 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 antibody-dependent cellular cytotoxicity mediated by macrophages elicited with bacillus Calmette-Guérin (BCG) [133]. Perhaps of more interest is the study of West Nile virus, which has been advanced as a model for Dengue haemorrhagic fever. The infectivity of West Nile virus, a flavivirus which can replicate in cells of the $P388D_1$ mouse macrophage cell line, is increased 100-fold by sub-neutralizing 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 [134]. 2.4G2 IgG has also been reported to act as a B cell mitogen and to stimulate a polyclonal antibody response [135]. The authors have, however, been unable to confirm these observations [unpublished results]. Indeed, Phillips & Parker [161] found no stimulatory activity of 2.4G2 *per se* on B cell activation, measured by the secretion of Ig. However, they did find that intact anti-mouse Ig antibodies, in the presence of 2.4G2 IgG, would induce B cell differentiation as efficiently as the $F(ab')_2$ fragments. The $Fc_\gamma R$ must apparently be directly cross-linked to the bound anti-μ chain to result in inhibition of B-cell differentiation.

Another area in which monoclonal antibodies have provided useful probes is in the study of induction and regulation. Hamburg *et al.* [136] demonstrated that although there was enhancement of macrophage phagocytosis of IgG opsonized E following 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.* [137] report that IgG2a binding, but not IgG2b binding, is selectively enhanced following stimulation of mouse macrophages by BCG. Yoshie *et al.* [138] find evidence for increased levels of both $FcR_{\gamma 2a}$ and $FcR_{\gamma 2b/\gamma 1}$ following induction with α and β interferon of the mouse macrophage cell line RAW 309 Cr.1. Fertsch & Vogel [162] report that cloned α, β, and γ-interferon are all capable of inducing $Fc_\gamma R$ on mouse macrophages, as demonstrated by increases of up to tenfold in the

binding and phagocytosis of IgG-coated sheep erythrocytes.

One of the primary functions of the $Fc_{\gamma}R$ in the reticuloendothelial system is the binding and clearance of immune complexes. Blockade of $Fc_{\gamma 2b/\gamma 1}R$ by 2.4G2 IgG should, therefore, affect the sequestration of immune complexes. Kurlander *et al.* [163] observed in mice that doses of 1–2 μg/g body weight of 2.4G2 IgG inhibited clearance in the liver of human serum albumin (HSA) rabbit anti-HSA complexes by 50%. There was no effect on the clearance of uncomplexed anti-HSA IgG. The Fab fragment of 2.4G2 IgG was 50% as effective in blockade of hepatic sequestration as the intact antibody. These observations have obvious import in the possible treatment of diseases in which immune complexes play an important role, such as lupus erythematosus, autoimmune haemolytic anaemia, and idiopathic thrombocytopenic purpura.

It is not clear whether the different mouse Fc receptors have specialized physiologic function. Ralph *et al.* [139] found that all IgG subclasses in mouse mediate phagocytosis and lysis of IgG-coated E. However, Ezekowitz *et al.* [137] found that IgG2a complexes more efficiently stimulated the oxidative burst of BCG-activated macrophages. Further suggestion that $Fc_{\gamma 2a}R$ may be of particular interest in tumoricidal/microbicidal activity comes from Matthews *et al.* [140], who found that macrophage cytotoxicity and *in vivo* protection against the 775 murine adenocarcinoma cell line was mediated by mouse IgG2a antibody. Supporting the hypothesis that different receptors have different functions, Nitta & Suzuki [141] find differences in cyclic nucleotide responses after adherence of IgG2a and IgG2b sensitized E. Adams *et al.* [164] have used a model of human colorectal carcinoma in nude mice to study the efficiency of different monoclonal anti-tumour antibodies to inhibit tumour growth, and found, echoing the results of Matthews *et al.* [140], that IgG2a but not IgG2b antibodies are effective. They suggest that IgG2a anti-tumour antibodies elicit macrophages activated for a novel antibody-dependent cellular cytotoxicity differing from that normally assayed in that long incubation times are required. The mechanism of the cytotoxicity, and the details of the activation of the macrophages are clearly topics of great interest.

Human Fc receptor

The analysis of human Fc receptors has largely focussed 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 [142] and Unkeless *et al.* [143]). 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 [144]. However, Messner & Jelinek [145], and Huber *et al.* [146] reported lack of binding of some anti-Rh_0 sera to neutrophils, suggesting a possible difference between neutrophil and monocyte receptors. These results have been confirmed recently by Kurlander & Batker [147], who demonstrated that human IgG1 oligomers bound with 100–1000-fold higher avidity to monocytes than to neutrophils. The neutrophil receptor is thus a relatively low-avidity receptor which probably is triggered only by immune complexes, in comparison to the monocyte receptor, which binds IgG1 monomer with a $K_a > 10^8$ M^{-1}.

Kurlander *et al.* [165] have extended the studies of IgG1 dimer binding to analyse human monocytes and peritoneal macrophages. Although the binding affinity of monomer IgG1 to macrophages and monocytes was comparable, Scatchard analysis of the binding of dimers to the macrophages revealed a marked curvilinearity not seen in the analysis of the binding of IgG1 dimers to monocytes. The data were explained by the presence of two classes of binding site on macrophages. The low affinity binding compartment was much larger than the high affinity compartment (218 000 ± 127 000 vs 42 000 ± 33 000 sites). The association constants for IgG1 dimers binding to the low and high avidity sites were 1.1×10^7 M^{-1} and 2.7×10^9 M^{-1}, a difference of over 200-fold. These results, as well as the isolation of monoclonal antibodies directed against the low avidity $Fc_{\gamma}R$, are consistent with the presence of at least two $Fc_{\gamma}Rs$ on human leucocytes, one of high affinity ($Fc_{\gamma}R_{hi}$) and one of low affinity ($Fc_{\gamma}R_{lo}$), present on macrophages and PMN.

There may be yet other $Fc_{\gamma}Rs$ specific for B lymphocytes and non-B lymphocytes. Cohen *et al.* [166] report different patterns of iodinated proteins following affinity chromatography of lysates from adherent cells, B cells, and B cell depleted lymphocytes on human IgG-Sepharose. Kulczycki [167], using similar methodology, found different Fc-binding proteins from human eosinophils and neutrophils. The neutrophil Fc-binding protein had a Mr of 52 000–68 000, and the eosinophil a Mr of 43 000. The neutrophil Fc_{γ} receptor rebound to IgG1-Sepharose but not IgG3-Sepharose, in contrast to the eosinophil receptor, which bound to both ligands.

Analysis of human Fc receptors using monoclonal antibodies has provided solid evidence for human Fc receptor heterogeneity. Fleit *et al.* [54] have isolated an anti-neutrophil Fc receptor monoclonal antibody, 3G8, by screening hybridoma supernatants for inhibition of neutrophil rosetting with IgG-sensitized erythrocytes. The Fab fragment of 3G8 retained its

potent inhibitory capacity against neutrophil Fc receptor. Immunoprecipitation of labelled neutrophils by 3G8 Fab-Sepharose revealed two poorly resolved peptides of Mr 53 000 and 66 000, which resemble the Fc receptor from mouse macrophages immunoprecipitated with 2.4G2 Fab-Sepharose. Affinity chromatography on IgG-Sepharose of ^{125}I-surface-labelled detergent lysates from human mononuclear cells and neutrophils resulted in a broadly-migrating protein of Mr 52 000–64 000 [148]. Comparable experiments using lysates of the U937 cell line resulted in molecules of 72 000 and Mr 40 000–43 000 [149].

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 [144,150]. 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 cultured *in vitro* for seven days, suggests that the neutrophil Fc receptor for IgG ($Fc_\gamma R_{lo}$) is either an inducible protein or a marker of a particular stage in the monocyte–macrophage differentiative pathway.

The authors have used the induction of the $Fc_\gamma R_{lo}$ receptor on HL-60 following treatment with retinoic acid or dimethylsulphoxide (DMSO) and patients with chronic myelogenous leukaemia (CML) to study the expression of neutrophil $Fc_\gamma R_{lo}$ during differentiation. Immunofluorescent staining of bone marrow cells for $Fc_\gamma R_{lo}$ and counter-staining for nuclear morphology with *p*-phenylenediamine, a free radical scavenger used to block fluorescence bleaching [151], revealed staining on cells at the metamyelocyte or later stages but not on less differentiated forms [unpublished results]. In agreement with these observations the uninduced HL-60 cell line, which has the morphological appearance of cells at the promyelocyte stage of differentiation, did not express the 3G8 antigen. However, after induction with DMSO or retinoic acid, both of which have been shown to drive HL-60 to more mature myeloid stages [152], from 5 to 40% of the cells synthesize $Fc_\gamma R_{lo}$. Finally, although the more mature cells in the peripheral circulation of patients with chronic myelogenous leukaemia have the same number of 3G8 binding sites, and bind the same amount of IgG in immune complexes as do peripheral neutrophils, immature cells from CML patients are completely negative for 3G8 antigen [168].

In the course of screening for monoclonal antibodies reactive with NK cells Perussia *et al.* [153,154] isolated an antibody, B73.1, which is probably directed against the same FcR as is monoclonal 3G8, isolated by Fleit *et al.* [54]. The antigens immunoprecipitated by the two antigens appear identical. B73.1 has a relatively low avidity, as demonstrated by the rapid dissociation of bivalent IgG or $F(ab')_2$, but B73.1 $F(ab')_2$ at 100 μg/ml does inhibit rosette formation with IgG-sensitized erythrocytes on NK cells by 93%. Incubation with B73.1 results in parallel decreases in B73.1 reactivity and FcR activity and, conversely, immune complexes down-modulate B73.1 antigen. The cellular distribution of the antigens recognized by B73.1 and 3G8 is similar in that monocytes, immature myeloid cells, and myelomonocytic cell lines are negative. These similarities are striking; thus it appears likely that both antibodies are reacting with the same Fc receptor.

There are, however, significant and intriguing differences between the two reagents, which suggest that different epitopes are being recognized. B73.1 binds to neutrophils of only half of normal donors, and is not present on eosinophils or B cells. In contrast, no polymorphism of the 3G8 antigenic site has been detected; 15% of B cells and all eosinophils are stained by 3G8 Fab. Interestingly, although B73.1 reactivity is found on neutrophils of half of the donors, it still binds to NK cells of all donors. The structural basis of these differences in specificity is not known, but it is possible that the polymorphism Perussia *et al.* [153,154] observe is related to post-synthetic modification of the protein.

Perussia & Trinchieri [169] reported that monoclonal antibody 3G8 also reacts with natural killer cells and the same peripheral lymphocyte subsets as does B73.1. The properties of B73.1, 3G8, Leu-11a, Leu-11b, and VEP-13, all of which are directed against $Fc_\gamma R_{lo}$, were examined by Perussia *et al.* [170]. VEP-13 and Leu-11b, since they are both IgM, can be used to lyse cells with complement. 3G8 was most efficient at blockade of function, and B73.1 had the advantage, in some subjects, of being unreactive with PMN. Miedema *et al.* [171] have also isolated an anti-$Fc_\gamma R_{lo}$ monoclonal antibody, CLB-FcR/1 with similar inhibitory properties as 3G8.

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 that, 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 the following: 2D2C, which immunoprecipitates a glycoprotein of Mr 90 000 and recognizes an alloantigen present on DBA/2, BALB/c, and CBA but not A, B10, B10.D2 or AKR mice [97]; 1.21J, which recognizes Mac-1, thought to be the CR_3 receptor [6]; 2E2A, which recognizes a protein of Mr 82 000; F4/80, which immunoprecipitates a macrophage-specific glycoprotein of Mr 150 000 [14]; 2F44, which recognizes a protein of Mr 42 000; 25-1, which recognizes H-$2D^d$; and 2.6, which immunoprecipitates a protein of Mr 20 000 [2]. 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 to the distribution in vesicles which were labelled following pinocytosis of lactoperoxidase [2]. In most cases, the relative distribution of proteins labelled on the plasma membrane was the same as the distribution of proteins in the labelled vesicles, and argues against exclusion of these proteins in pinocytic vesicles. However, one protein, recognized by monoclonal antibody 2.6, was preferentially represented in the labelled pinosome proteins relative to the plasma membrane [2]. Muller *et al.* [130] have examined the protein composition of endocytic vacuoles formed by macrophage phagocytosis of latex particles, and they find that 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 [97]. 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.

In patients with circulating immune complexes, there is often a defect in the rate of clearance of IgG-coated erythrocytes [155]. 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.* [156] studied the rate of degradation of $Fc_{\gamma 2b/\gamma 1}R$ after phagocytosis of erythrocyte ghosts coated with IgG, and found a significantly increased rate of degradation ($T_{\frac{1}{2}} < 2h$) relative to that of the free receptor ($T_{\frac{1}{2}} = 10$ h). 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 h. Thus the ligand can profoundly affect the subsequent turnover and degradation of $Fc_{\gamma 2b/\gamma 1}R$ while not altering the turnover and recycling of other plasma membrane proteins.

The fate of $Fc_{\gamma 2b/\gamma 1}R$ tagged with monovalent 2.4G2 Fab fragment has been examined and, in contrast to the results with opsonized erythrocyte ghosts [155], the receptor appears to recycle [172]. The internalized $Fc_{\gamma 2b/g1}R$–^{125}I–2.4G2 Fab complex was distinguished from complexes on the cell surface by resistance of the internalized complex to dissociation by mild (pH 4) acid. Recycling from a low density endosomal compartment was only slightly affected by monensin or NH_4Cl, and the ^{125}I–2.4G2 Fab was only slowly degraded. However, Mellman & Plutner [173] report that soluble immune complexes are efficiently taken into lysosomes and degraded to TCA-soluble products, with concomitant selective removal of $Fc_{\gamma 2b/g1}R$, which is then degraded. These results are in substantial agreement with previous studies by Segl *et al.* [174,175], who studied the endocytosis of aggregating and non-aggregating oligomers of rabbit IgG. Aggregating dimers were rapidly cleared from the cell surface of P388D1 mouse macrophages, and $\frac{2}{3}$ of the cell $Fc_\gamma R$ disappeared from the cell surface as a consequence of the endocytosis. The non-aggregating dimers were not efficiently internalized.

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