March 2011

FocusOn No.027

Antibodies for Human Macrophages

Blood monocytes are young cells that already possess migratory, chemotactic, pinocytic and phagocytic activities, as well as receptors for IgG Fc-domains (FcR) and iC3b complement. Under migration into tissues, monocytes undergo further differentiation to become multifunctional tissue macrophages. For many research projects it is important to differentiate cells of the monocyte/macrophage lineage. Some macrophage specific proteins and specific clones useful for such an application are presented below.

AIF-1

Allograft Inflammatory Factor-1 is a $Ca2^{+}$-binding peptide produced by activated macrophages and microglial cells. AIF-1, like the peptides MRP8 and MRP14, is expressed by activated macrophages and might participate in a variety of pathogenic processes in the mammalian brain and in chronic transplant rejection.

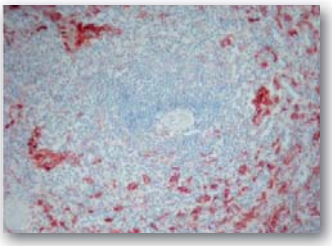

Fig. 1: *Staining of formalin-fixed, paraffin-embedded (FFPE) human spleen sections with AIF1 antibody* ***Cat.-No. BM4114***

CD163

Clone RM3/1 (BM4021) recognises the late stage inflammatory macrophages scavenger receptor (CD163), a membrane glycoprotein on human monocytes and macrophages which is expressed in the intermediate and late phase of inflammation. The antigen is a member of the scavenger receptor family. CD163 is significantly up-regulated by glucocorticoids while it is down-regulated by cyclosporin A and by phorbol esters. An important function of CD163 seems to be in the adhesion of monocytes to activated endothelial cells. RM3/1-positive cells include skin histiocytes, Kupffer cells, spleen macrophages of the red pulp, and some thymus macrophages. The antigen is found abundantly in human term placenta, and regularly in acute and chronic inflammatory lesions. A different epitope of the same antigen is recognised by antibody Ki-M8 (BM4112). RM3/1 is useful for macrophage phenotyping, for the classification of intermediate inflammatory stages in tissue sections and cell smears (together with clones 27E10 (BM4025) and 25F9 (BM4022)), for the characterization of tumorous tissues, and monitoring of macrophage cell cultures.

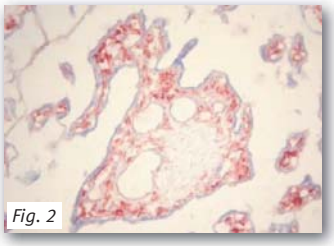

Fig. 2

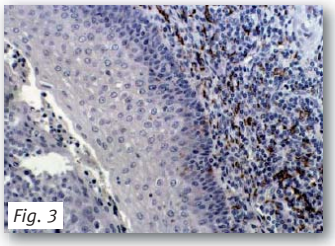

Fig. 3

Fig. 2: *Frozen human placenta, stained with CD163 clone RM3/1* ***Cat.-No. BM4021***
Fig. 3: *Staining of human tonsil sections (FFPE) with CD163 antibody clone 5C6-FAT* ***Cat.-No. BM4041***

CD68

CD68 could play a role in phagocytic activities of tissue macrophages, both in intracellular lysosomal metabolism and extracellular cell-cell and cell-pathogen interactions. Binds to tissue- and organ-specific lectins or selectins, allowing homing of macrophage subsets to particular sites. Rapid recirculation of CD68 from endosomes and lysosomes to the plasma membrane may allow macrophages to crawl over selectin-bearing substrates or other cells. Highly expressed by blood monocytes and tissue macrophages CD68 is also expressed in lymphocytes, fibroblasts, endothelial cells and in many tumor cell lines which could allow them to attach to selectins on vascular endothelium, facilitating their dissemination to secondary sites.

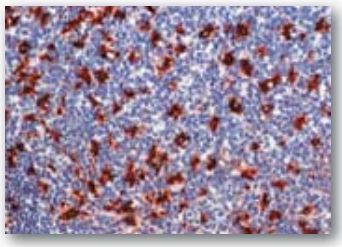

Fig. 4: *Human tonsil (FFPE) stained with CD68 antibody clone KP1* ***Cat.-No. DM167***

Tab. 1: *Cell reactivity pattern of some monocyte/ macrophage antibodies*

	Macrophages	Monocytes	Granulocytes	Lymphocytes	Dendritic C.	Histiocytes	Kupffer C.	Langerhans C.	Endothelial C.	Epithelial C.
25F9	+					+	+			
27E10	±	+	±							
3A5	+	+	±	-	-	+		-		
5C6-FAT	±	+				+	+			
8-5C2	±	+	+							
Ki-M7	+	+	+			±				+
Ki-M8	+	+				±				+
MAC387	+	+	+							
PM-2K	+				-		+	-		
R20	+	+								
RFD7	+	-	-							
RM3/1	+		-			+	+			
S13.67	±	+	+							
S32.2	±	+	+							
S36.48	±	+	+							
X-4	+	-	-				+			
X-14	+	-	-				+			
YTH.18	+	±	+					-		

MRP8/14 (S100A8/S100A9)

There are several antibodies available now to detect MIF Related Proteins [MRP; Clones 27E10 (BM4025), MAC387 (SM2011P), 8-5C2 (BM4028), S13.67 (BM4029), S36.48 (BM4026) and S32.2 (BM4027)], which are extremely useful in the analysis of several disease conditions, particularly in the inflammatory diseases and certain microbial infections, as well as neoplastic conditions. Clinically relevant is especially the detection of MRP8/14 in certain autoimmune conditions and transplant rejection. MRP8 (Calgranulin A, 10.8 kDa) and MRP14 (Calgranulin B, 13.2 kDa) belong to the S-100 protein family (S100A8 and S100A9) and can form Ca2+ dependent homo- or hetero-complexes of various composition. The MRP8/14 heterocomplex is also known as Calprotectin, L1 Protein (not related to the neural adhesion molecule L1) and cystic fibrosis antigen. MRP8/14 is found in cells, tissues, and fluids in all parts of the human body. It is mainly a myelomonocyte and keratinocyte protein. In neutrophils MRP8/14 is located in the extralysosomal cytosol in concentrations estimated at 5-15 mg/ml, which corresponds to about 5% of total proteins in neutrophils. Numerous biological functions can be attributed to MRP8 and 14. The S-100 like structure and the calcium dependent association to cytoskeleton structures suggest intracellular signal transduction functions. The antimicrobial function of MRP8/14 has been well documented. While MRP8 is chemotactic for neutrophils, MRP14 seems to have just the opposite effect. Most important however, the presence of MRP8/14 in acute, and its absence in chronic inflammation is an excellent tool in assessing a vast number of pathological conditions.

Clone YTH 8.18

is reactive with all tissue macrophages, binding to a cytoplasmic epitope. In peripheral blood the antibody stains all granulocytes and around 50% of monocytes. No staining of osteoclasts or Langerhans' cells is seen.

Clone RFD7

recognises an antigen which is predominantly expressed cytoplasmically in mature tissue phagocytes. RFD7 is co-expressed with RFD1 on suppressive macrophages. RFD7 does not react with circulating monocytes or any cells within the granulocyte series.

Clones PM-2K, X-4 and X-14

form a particular group of macrophage specific antibodies which were classified at the V Leukocyte Typing Workshop held in Boston in 1993. PM-2K recognises tissue macrophages and macrophages in proliferative disorders of macrophages. PM-2K is one of the markers that, being negative on dendritic cells, can differentiate between macrophages and dendritic cells.

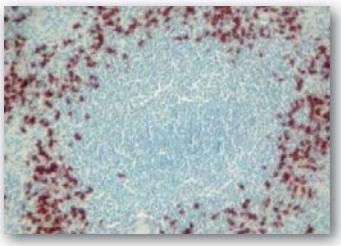

Fig. 5: Immunohistochemical staining of human spleen sections (FFPE) using S100A9 antibody clone S36.48 ***Cat.-No. BM4026***

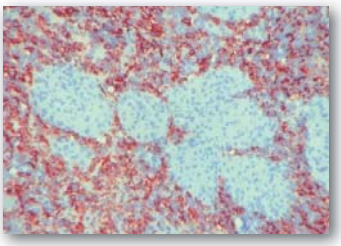

Fig. 6: Staining of swine spleen frozen sections using macrophages antibody clone PM-2K ***Cat.-No. BM4037***

Name	Host/Isotype	Clone	Reactivity	Application	Catalog-No.
AIF1	Goat IgG	-	Hu, Ms, Rt	E, P, WB	AP08912PU-N
AIF1	Goat	-	Hu, Ms, Por, Rt	E, WB	AP16011PU-N
AIF1	Goat IgG	-	Can, Hu	E, P, WB	AP08793PU-N
AIF1	Goat	-	Can, Hu, Ms, Rt	P, WB	AP16467PU-N
AIF1	Goat	-	Can, Hu	P, WB	AP16584PU-N
AIF1	Mouse IgG2b	1022-5	Hu, Rt	P	BM4114*
CD163	Rabbit IgG	K20-T	Hu	P	AM10011PU-S
CD163	Mouse IgG1	R20	Hu	E, F, WB	BM2518
CD163	Mouse IgG1	RM3/1	Hu, Mky	C, F, WB	BM4021
CD163	Mouse IgG1	5C6-FAT	Hu	C, E, F, P, WB	BM4041*
CD163	Mouse IgG1	Ki-M8	Hu	C, F, WB	BM4112
CD68	Mouse IgG1	Ki-M7	Hu	C	BM4111*
CD68	Mouse IgG3	PG-M1	Hu	P	DM094
CD68	Mouse IgG1	KP1	Hu	P	DM167
CD68	Rabbit	-	Hu	WB	AP19034PU-N
MRP8/14 (S100A8/A9)	Mouse IgG1	27E10	Hu	C, E, P	BM4025*
MRP8/14 (S100A8/A9)	Mouse IgG1	MAC387	Bov, Can, Eq, Fe, GP, Hu, Mky, Por, Rb, Rt	C, F, P	SM2011P*
MRP14 (S100A9)	Mouse IgG1	S36.48	Hu	C, E, F, P	BM4026*
MRP14 (S100A9)	Mouse IgG1	S32.2	Hu	C, E, F, P	BM4027*
MRP8 (S100A8)	Mouse IgG1	8-5C2	Hu	C, E, P, WB	BM4028*
MRP8 (S100A8)	Mouse IgG1	S13.67	Hu, Por, Rt	C, E, F, P, WB	BM4029*
Macrophages	Mouse IgG1	25F9	Hu	C, P	BM4022*
Macrophages	Mouse IgG1	X-4	Hu	C	BM4035
Macrophages	Mouse IgG1	X-14	Hu	C	BM4036
Macrophages	Mouse IgG1	PM-2K	Hu	C	BM4037
Macrophages / Neutrophils	Rat IgG2b	YTH8.18	Hu	C	SM1218
Macrophages / Histiocyten	Mouse IgG1	RFD7	Hu, Mky	F	SM1216
Macrophages / Histiocyten	Mouse IgG2b	3A5	Hu	C, P	SM1219

Can: Canine, Eq: Horse, Fe: Feline, GP: Guinea pig, Hu: Human, Mky: Monkey, Ms: Mouse, Por: Pig, Rb: Rabbit, Rt: Rat

C: Immunohistochemistry on frozen sections, E: ELISA, F: Flow cytometry, P: Immunohistochemistry on formalin-fixed, paraffin-embedded tissue sections, WB: Western blot

* Conjugates available