SUPPLEMENTARY INFORMATION (for online publication only)

CARBOXYPEPTIDASE-M IS REGULATED BY LIPIDS AND CSF IN MACROPHAGES AND DENDRITIC CELLS AND EXPRESSED SELECTIVELY IN TISSUE GRANULOMAS AND FOAM CELLS

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METHODS

Supplementary information for Carboxypeptidase-M (CPM)

Immunohistochemistry (IHC)

To check the specificity, positive and negative controls were included for each immunohistochemistry run. Negative controls for CPM and the reference macrophage markers consisted of slides with isotype-matched control mAB IgG (Dako-Cytomation) in place of the primary antibody[11]. For all immune-peroxidase stainings, the nuclear counterstainings were with methyl-green or hematoxylin. Normal placenta served as a positive control for CPM-immunostainings where trophoblasts show membrane positivity (Figure S1-A). The immune-stained specimens for macrophage labeling were judged semi-quantitatively as no staining (0); weak staining (1+); moderate staining (2+); intense staining (3+).

Supplementary information for monocyte separation

Cell viability was at least 98% as determined by trypan-blue exclusion, and monocyte purity was varied between 95%-98% as judged by morphology and flow cytometry analysis for CD14 expression. Separated cells were resuspended (1.5×10^6/ml) in

RPMI1640 supplemented with 10% heat inactivated fetal bovine serum (FBS) (Invitrogen), 500 U/ml penicillin-streptomycin and 2mM L-glutamine (Gibco). For cell culturing $25cm^3$ tissue culture flasks (TPP; Trasadingen, Switzerland) were used. All cultures were maintained in a humidified 37°C incubator venting 5% CO_2. [28-29]. The cells were grown on coverslip for in situ immunofluorescence combined with nile-red lipid staining.

Supplementary information for macrophage-infection with mycobacteria

Mycobacterium bovis-BCG tuberculosis bacilli (TB) was purchased in lyophilized form (Statens Serum Institut, Copenhagen, Denmark) and dissolved in culture medium right before infection, and clumps were removed by a 10 minutes centrifugation at 20 g. The density of bacteria in the cultures was adjusted to a dose of 1 bacterium/cell assuming that an OD 600 of 0.2 equals a concentration of 10^7 bacteria/ml. At the indicated time points, the cells were collected with centrifugation, and the pellets were then subjected to formalin fixation, paraffin embedding and sectioning for immuno-fluorescence staining as earlier described[29].

Statistical analysis

In real-time quantitative RT-PCR experiments, the mean and SD were calculated for both the normalized and the normalizer values. To incorporate the random errors of the measurements, we used the propagation of errors to determine the SD of the normalized values. We made at least three biologic replicates for all experiments. In real-time quantitative PCR experiments, we performed an unpaired, two-tailed, *t* test. $p < 0.03$ was considered statistically significant.

SUPPLEMENTARY RESULTS

The sensitivity and specificity of MA-CPM IHC on routinely processed tissues

The IHC findings for carboxypeptidase-M expression in macrophages are summarized in supplementary Table S1. By comparing the CPM expression patterns with CD68 and CD163 on tissue sections, it is obvious that CPM detects only small subpopulation(s) of macrophages (i.e., epithelioid and lipid-laden cells, only), as opposed to reference markers which decorate virtually any types of macrophages (Figure 1B, CD163 vs. CPM; Figure 2B CD68 vs. CPM; Figure 3 C-D, reference markers vs. CPM). Therefore, the sensitivity of CPM for macrophage detection is low when used as a universal (pan-)macrophage IHC-marker on paraffin tissue sections. However, the marker CPM appears to be highly specific and sensitive for the detection of EPCs and lipid-laden macrophages, respectively. To allow specificity, no reactivity was seen in normal condition in adipose tissues, fatty changed hepatocytes, non-activated Kuppfer cells of the liver (not shown), as well as in resting macrophages, “regular” non-specific inflammatory cells and alveolar macrophages e.g., associated with congestive hear failure (supplementary Figure S1), persistent chronic inflammation with no significant cell damages. Also, within normal lymph nodes, basically no CPM^+ “resting” sinus histiocytes are observed unless lipid uptake or cytokine-effects induced foamy or antigen-presenting dendritic cells, e.g., due to the draining components of tumor cells to the lymph node (Fig. 8C-D).

Technical notes for CPM-IHC in paraffin sections versus cryosections

Due to lipid-association of CPM-binding to cell membranes[6-7,13-14] and the use of organic solvents (acetone, xylene, ethanol) during paraffin tissue processing (which partially removes the lipids along with the majority of the coupling proteins), the CPM immune-staining intensities may decrease in paraffin-sections. Nevertheless, the positive staining can still clearly be recognized on commercially processed paraffin tissues (Figure 7A-C), but cryosections proved to be more effective in some cases (not shown).

Table 1. List of human cases that were investigated for comparative CPM-immuno-labeling. [1], not relevant; [2], number of positive cases; [3], range of dominant staining intensities in positive cases; [4], calcified plaques with no detectable macrophages; **EPCs**, epitheloid cells; **MAs**, macrophages; **TAM**, tumor-infiltrating macrophages.

Lesion type	**Case number**	**EPCs**		**Non-granulomat MAs**	
	n	**CPM**	**CD68/CD163**	**CPM**	**CD68/CD163**
Inflam, no tissue damage	20	NR[1]	NR	0	[2]20(2-3[3])
Inflam, tissue damage	20	NR	NR	6(1-3)	20(2-3)
Granulomatous-epithelioid lesions					
Sarcoidosis	48	[2]48(1-3[3])	42(1-3)	0	48(2-3)
Early Tuberculosis	4	4(2-3)	4(2-3)	0	4(2-3)
Adv Tuberculosis	16	3(0-2)	16(1-3)	0	16(1-3)
BCG-lymphadenitis	2	0	2(1-3)	1(1-2)	2(1-3)
Granulomat interstit pneum	6	6(1-3)	6(2-3)	0	6(1-3)
Wegener granulomatosis	6	4(1-3)	6(2-3)	2(1-3)	6(1-3)
Foreign body reaction	10	10(1-3)	10(2-3)	0	10(1-3)
Infective granulomas	7	6(1-3)	7(2-3)	0	7(2-3)
Toxoplasma lymphadenitis	6	6(2-3)	5(2-3)	0	6(2-3)
Cat scratch dis lymph node	5	4(1-2)	5(2-3)	0	5(2-3)
Amyloidosis with granuloma	2	2(1-2)	2(2-3)	0	2(2-3)
Gouty tophus	4	4(1-3)	4(2-3)	0	4(2-3)
Rheumatoid nodules	5	3(1-2)	5(2-3)	0	5(2-3)
Panniculitis granulomat	3	3(1-2)	3(2-3)	1(1-2)	3(1-3)
Granuloma annulare	3	3(1-2)	3(2-3)	0	1(1-3)
Granuloma faciale	2	2(1-2)	2(1-3)	0	2(1-3)
Solar dermatosis, granulomat	3	3(1-3)	3(2-3)	0	3(2-3)
Crohn's dis. with granuloma	4	4(1-3)	3(1-3)	0	4(1-3)
Crohn's dis, no granuloma	8	NR	NR	0	8(1-3)
PBC with granuloma	4	2(1-3)	4(1-3)	0	4(1-3)
Lesions with lipid-laden MAs		**Foam cells**		**Non-foam MAs**	
Cholesterosis (gallbadder)	12	12(1-3)	12(2-3)	0	12(1-3)
Early atheroscler plaque	7	6(1-2)	7(2-3)	0	7(1-2)
Adv atheroscler plaque[4]	5	NR	NR	0	3(1-2)
Xanthelasma	3	3(1-3)	3(2-3)	0	NR
Lipid granuloma	10	10(1-3)	10(1-3)	0	10(2-3)
Gaucher disease, typeI	4	4(2-3)	4(2-3)	0	4(1-3)
lipid pneumonia	3	3(2-3)	3(2-3)	0	3(2-3)
Others					
TAM with tu-cell damage	7	5(2-3)	7(2-3)	0	7(2-3)
TAM without tu-cell damage	7	0	7(2-3)	0	7(2-3)
Total cases	**246**				