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Inducible Expression of MS-1 High-Molecular-Weight Protein by Endothelial Cells of Continuous Origin and by Dendritic Cells/Macrophages *in Vivo* and *in Vitro*

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Recently, we have described a monoclonal antibody, named MS-1, which identifies a novel high-molecular-weight protein expressed by noncontinuous, sinusoidal endothelia and by interstitial dendritic cells in certain normal human organs (S Goerdt, LJ Walsh, GF Murphy, JS Pober, J Cell Biol 1991, 113:1425–1437; and LJ Walsh, S Goerdt, JS Pober, H Sueki, GF Murphy, Lab Invest 1991, 65: 732–741). In this report, we demonstrate in studying a variety of skin lesions that MS-1 antigen can also be expressed by endothelia of continuous origin under certain pathological conditions. Among the skin lesions tested, MS-1 antigen expression by endothelial cells of continuous origin is frequently observed in wound healing tissue, in cutaneous T-cell lymphoma, in psoriasis, and in melanoma metastasis, ie, in 100%, 80%, 71%, and 71% of cases, respectively. In contrast, endothelial MS-1 antigen expression rarely occurred in other skin lesions, including vascular tumors, six of which were Kaposi's sarcomas (13% and 0% of cases with vascular MS-1 expression, respectively). The percentage of cases with MS-1$^+$ vessels is only marginally different in malignant* versus *benign lesions (55%* versus *31%); when melanocytic nevi, primary melanomas, and melanoma metastases are compared, however, an increase in the percentage of cases with MS-1$^+$ vessels is seen (31%, 50%, and 71%, respectively). Apart from wound healing, the relative number of MS-1$^+$ vessels in a given lesion amounts to less than 5% compared with the number of continuous type vessels stained by monoclonal antibody 1F10 (S Goerdt, F Steckel, K Schulze-Osthoff, H-H Hagemeier, E Macher, C Sorg, Exp Cell Biol 1989, 57: 185–192). In addition, the occurrence of MS-1$^+$ vessels is not related to the overall vascularity of a given lesion. Thus, the conditions for MS-1 antigen expression by endothelia of continuous origin cannot as yet be exactly defined. Furthermore, we have noticed that the number of MS-1$^+$ dendritic cells varies considerably in skin lesions; in the early patch lesions of Kaposi's sarcoma and in juvenile xanthogranuloma MS-1$^+$ cells even constitute the major cell type. This prompted us to investigate MS-1 antigen expression and its regulation in cultured human monocytes/macrophages. Expression of MS-1 antigen by these cells regularly starts at day 3 of culture and reaches its maximal value at day 9, after which it declines. Of the mediators tested—γ-interferon (IFN-γ), tumor necrosis factor α, interleukin-1β (IL-1β), IL-4, IL-6, granulocyte-monocyte colony-stimulating factor, monocyte colony-stimulating factor, and dexamethasone—IL-4 and dexamethasone (singly or in combination) exert the strongest inductive effects. IFN-γ strongly inhibits unstimulated and IL-4-sustained MS-1 antigen expression, while it is much less effective in inhibiting dexamethasone-induced MS-1 antigen expression. Immunoprecipitation from IL-4- and dexamethasone-* versus *IFN-γ-treated monocytes confirms that 1) MS-1 protein expression can be readily suppressed or sustained by IFN-γ or by IL-4 and glucocorticoid, respectively, and 2) that MS-1 antigen in macrophages consists of three protein species of 320, 280, and 220 kd. (Am J Pathol 1993, 142:1409–1422)

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Endothelial cells and dendritic cells are both cell types that comprise heterogeneous cell populations in various anatomic locations. Different methodical approaches such as histology, histochemistry, immunohistochemistry, and electron microscopy have been used to study the phenotypes, structural requirements, and functions of these cells. With regard to dendritic cells, which are now considered to be of bone marrow origin and related to macrophages, it has been possible to more closely define epidermal Langerhans cells (CD1+), follicular dendritic cells, and interdigitating reticulum cells as distinct subpopulations.[1–3] Other putative subpopulations of dendritic cells such as dermal Langerhans cells/indeterminate cells, dendritic cells/veiled cells in afferent lymphatics, sinus histiocytes, histocytic reticulum cells, and factor XIIIa$^+$ dendritic cells (also called perivascular dendritic macrophages and/or dermal dendrocytes) are less well characterized.[2,4–11] Most dendritic cells are thought to represent unique types of very efficient antigen presenting cells. In contrast, the function of the factor XIIIa$^+$ dendritic macrophages[12,13] or the sinus histiocytes is less clear. With regard to endothelium, it has been possible to underscore the significance of the morphological classification in continuous (most blood vessels) and discontinuous (sinusoids and lymphatic vessels) endothelia with the help of monoclonal antibodies (mAbs) identifying molecules which are differentially expressed in these endothelia. Continuous endothelia, for example, selectively express the endothelial cadherin,[14] CD34,[15–17] CD31,[18–20] and 1F10 antigen.[21] The latter two molecules are subject to modulation of their expression by extracellular matrix components: CD31 relocates to the junctional complexes when endothelial cells are cultured on rehydrated collagen,[19] and the 1F10 antigen is only weakly expressed in cultured endothelial cells unless they are placed on a basement membrane-like substrate such as Matrigel (S. Goerdt and J. S. Pober, unpublished observations). In addition, evidence is accumulating showing a close antigenic relationship between dendritic cells, in particular perivascular dendritic cells, and endothelial cells in that they may share expression of M241 membrane glycoprotein,[22] CD34,[23,24] and CD36.[25–29]

Recently we have described and characterized a mAb, named MS-1, which was raised against human spleen and detects a high-molecular-weight protein antigen expressed selectively by noncontinuous, sinusoidal endothelial cells in spleen, liver, and lymph node and by interstitial dendritic cells in placenta, gut, and skin.[30] Immunoprecipitation, pulse-chase experiments and limited proteolysis peptide mapping showed that the MS-1 antigen consists of four protein species, ie, a 280 kd precursor which is converted to a 300/320 kd mature form which, in turn, is partially cleaved to 205/220 kd and 120 kd species. The MS-1 protein species are soluble in aqueous buffer and are secreted *in vitro*. MS-1 protein is different from the well-known large extracellular matrix proteins von Willebrand factor, tenascin, laminin, and fibronectin and from a number of other endothelial and dendritic antigens. In splenic endothelium, MS-1 antigen is predominantly deposited at zones of membrane-membrane and membrane-matrix contact. In MS-1-positive, highly dendritic perivascular macrophages that express both HLA-DR antigens and factor XIIIa, the antigen is found in discrete cytoplasmic compartments (vesicles or membranous cisternae) and focally on the plasma membrane colocalizing with plasma membrane-asssociated plaques that interface with extracellular microfibrils.[31] These morphological, ultrastructural, and biochemical characteristics of MS-1 antigen all suggest that this molecule may serve an anchoring function for the structural maintenance of discontinuous endothelia that do not normally produce basement membranes or vessel walls and of highly dendritic perivascular macrophages with similar requirements as noncontinuous endothelial cells for spatial localization.

In ontogeny and under pathological conditions, endothelial cells may undergo changes in activation and differentiation, eg, acquire or lose continuity, become leaky, express adhesion molecules, synthesize cytokines, proliferate, and migrate. This phenomenon is most clearly seen in the formation of tumor vessels. Tumor vessels are, in general, rather contorted and leaky,[32,33] possibly under the influence of tumor-derived vascular permeability factors.[34–38] Endothelial cells in the vasculature of brain tumors[39,40] no longer display (blood-brain barrier-specific) characteristics of their parental endothelial cells and may even acquire characteristics of alternatively differentiated endothelia, such as fenestrations, while in other tumors vascular hyperpermeability need not necessarily be accompanied by overt morphological changes.[41] A similar process is at work in inflammatory processes such as psoriasis: papillary capillaries become dilated, tortuous, and hypertrophied and develop fenestrations.[42] Moreover, blood vascular endothelial cells during development of the vasculature[43–45] and undergoing inflammatory angiogenesis,[46] eg, at the surface of pyogenic granuloma,[45,47] may actually display a noncontinuous phenotype.

In line with these data, we herein show that MS-1 high-molecular-weight protein can be expressed by

endothelia of continuous origin in diseased skin. The pathobiological conditions of this phenomenon, however, cannot as yet be exactly specified. In addition, we find that MS-1 expression by dendritic cells and other macrophages is variable in skin lesions. MS-1 expression by cultured human monocytes/macrophages is under close control by cytokines (IL-4 versus IFN-γ) and glucocorticoid and may indicate a macrophage phenotype with distinctive immune functions.

Materials and Methods

Cells

The A375 human melanoma-derived cell line was kindly provided by Dr. Josef Brüggen and was propagated in Eagle's minimal essential medium with Earle's salts and with 0.85 g/L $NaHCO_3$, but without glutamine (catalogue no. F0313, Biochrom, Berlin, Germany) supplemented with 15% fetal calf serum (Biochrom) and appropriate concentrations of penicillin/streptomycin and glutamin. Human monocytes were purified using either heparin-anticoagulated blood from single donors or pooled buffy coats (Blutbank des DRK, Münster). Twenty-five ml of heparin-anticoagulated blood or buffy coat were layered on top of 20 ml Ficoll-Paque (Pharmacia, Freiburg, Germany) in a 50-ml centrifuge tube and were centrifuged for 40 min at 650 × *g* at room temperature. Mononuclear cells were collected at the serum/Ficoll interface and washed three times in Ca^{2+}-free Spinner's minimal essential medium (Biochrom). Three ml of a suspension of 2 × 10^8 mononuclear cells/ml in Spinner's minimal essential medium were layered on top of 30 ml of a preformed Percoll gradient (Pharmacia) in a 50-ml Percoll tube (Pharmacia); the Percoll gradient (27 ml Percoll, 3 ml 10× Eagle's minimal essential medium with Earle's salts and with 0.85 g/L $NaHCO_3$, without glutamine) had been preformed in a SS-34 rotor of an ultracentrifuge (Sorvall, Frankfurt, Germany) at 12,000 rpm for 12 min at 20 C. Percoll gradients with the cells on top were centrifuged at 650 × *g* for 40 min at room temperature. The upper layer of cells containing approximately 80–90% monocytes was collected, and the cells were washed three times in Spinner's minimal essential medium. For culture, monocytes were resuspended in McCoy's medium supplemented with 20% pooled human serum and appropriate concentrations of penicillin/streptomycin, glutamin, and nonessential amino acids and were then transferred into teflon-coated, ultraviolet-irradiated plastic bags (Biofolie, Heraeus, Hanau, Germany) which were sealed using Polystar 410 HM (Rische und Herfurth, Hamburg, Germany). Cell number varied between 0.2 and 2 × 10^6 monocytes/ml according to the length of the culture period (0–15 days). Incubation was at 7% CO_2. Before harvest, the plastic bags were put on ice for at least 30 min and lightly hit with a stick for a while to get the lightly adherent cells back into suspension. The bags were then cut open and the cells were washed and either frozen as pellets for biochemical purposes or used to prepare cytospin preparations (Shandon, Frankfurt, Germany). Mediators were added directly to the medium at the beginning of the culture period, or the bags were cut open, the mediators added in appropriate concentrations, and the bags resealed 24 hours before harvest.

Antibodies

Antibodies used in this study were mouse mAb MS-1 against a high-molecular-weight protein expressed in noncontinuous sinusoidal endothelial cells and in certain dendritic cells,[30,31] mouse mAb RM 3/1 against a subpopulation of monocytes/macrophages,[48,49] a mouse mAb against CD23[50] purchased from Dianova (Hamburg, Germany), and rat mAb 1F10 against a 150 kd antigen specifically expressed by continuous endothelia.[21]

Mediators

Recombinant human γ-interferon (IFN-γ), used at 10, 100, or 1000 U/ml, respectively; human recombinant tumor necrosis factor-α (TNF-α), used at 20 ng/ml; human recombinant interleukin-6 (IL-6), used at 200 U/ml; human recombinant interleukin-1β (IL-1β), used at 20 U/ml; and human recombinant granulocyte-monocyte colony-stimulating factor, used at 100 U/ml, were purchased from Boehringer Mannheim (Mannheim, Germany). Human recombinant monocyte colony-stimulating factor, used at 100 U/ml, was purchased from Genzyme (Boston, MA). Human recombinant interleukin-4 (IL-4), used at 300 U/ml, was purchased from Amersham (Braunschweig, Deisenhofen, Germany). Dexamethasone, used at 5 × 10^{-7} mol/L, was from Sigma.

Immunohistochemistry

Portions of human skin tissues, derived from discarded tissue removed at surgery, were snap-frozen

in liquid nitrogen and stored at −70 C. Frozen sections of 6–10-μm thickness were prepared with a Reichert Histostat 855 Cryostat Microtome, air-dried, and fixed for 10 min in acetone. Cytospin preparations of nonadherent cells were fixed similarly. Fixed slides were subjected to quenching of endogenous peroxidase in phosphate-buffered saline (PBS) containing 10 mmol/L sodium azide and 0.1% hydrogen peroxide for 15 min, followed by a preincubation step in 1% bovine serum albumin (BSA) (radioimmunoassay grade, Sigma) in PBS for 20 min. Primary antibodies, either as hybridoma culture supernatants or as concentrated antibody fluid or ascites diluted in 1% BSA in PBS, were applied for 30 min at room temperature. For double labeling two primary antibodies made in different species (mouse/rat) were mixed together in 1% BSA in PBS and applied simultaneously for 30 min. After three 3-min washes in PBS, an appropriate horseradish peroxidase-labeled secondary antibody (Jackson Immunoresearch, West Grove, PA), diluted 1:100 in 1% BSA in PBS, was applied for 30 min. For double labeling two appropriate double-labeling grade, species-specific secondary antibodies (Jackson Immunoresearch), the first peroxidase-, the second alkaline phosphatase-labeled, were each diluted together in 1% BSA in PBS to a final concentration of 1:50. After another washing step, peroxidase was developed with amino-ethyl-carbazole (130 mg/L) in 0.1 mol/L sodium acetate buffer for 10 min, followed for double labeling by alkaline phosphatase development with Fast Blue RR salt (240 mg/L) in 0.01% naphthol-AS-MX-phosphate buffer containing 600 mg/L levamisole for 30 min. Single-labeled slides were counterstained in Gill's hematoxylin. All slides were mounted in glycerol-gelatin. For analysis, 200 cells of a cytospin preparation were counted on a single cell basis by two investigators independently, allowing a decision between antigen-positive or antigen-negative cells only.

Immunoprecipitation

To immunoprecipitate from monocytes/macrophages (and A375 melanoma cells), cells were harvested by centrifugation (or by trypsinization and centrifugation, respectively) and washed three times in serum-free medium, and the cell pellet was snap-frozen in liquid nitrogen. Cells (1×10^8/lane) were then directly lysed in cold 10 mmol/L Tris-HCl (pH 7.4), 150 mmol/L NaCl buffer containing 2% Nonidet P-40 and appropriate concentrations of the protease inhibitors soybean trypsin inhibitor, leupeptide, and aprotinin (all from Sigma). Lysates were incubated on a rotator for 1 hour and centrifuged for 20 min at 12,000 × *g* in an Eppendorf centrifuge at 4 C. Five μl of primary antibody (ascites) were added to 500 μl of lysate. Primary antibody incubation continued for 2 hours at 4 C. Immune complexes were collected on species-specific anti-immunoglobulin Sepharose 4B beads (Organon Teknika, Malvern, PA) for 2–4 hours at 4 C. The beads were washed ten times in 0.1 mol/L Tris-HCl (pH 8.0) with 0.2% Nonidet P-40 at room temperature, and the antigen was eluted by boiling in reducing sodium dodecyl sulfate sample buffer. Eluted antigen was electrophoresed on a 5% polyacrylamide gel at 6.5 mA constant current for 18 hours in the Laemmli buffer system; under these conditions, the front migrates off the bottom of the gel, achieving optimal resolution in the high-molecular-weight range. The gel was fixed with 30% ethanol and 10% acetic acid and silver stained (2 cycles, as recommended by the protocol) using a kit obtained from Sigma.

Table 1. *Expression of MS-1 Antigen in Endothelial Cells of Continuous Origin Compared to the Mean Vascularity of the Lesions as Measured by 1F10⁺ Vessels*

Diagnoses	No. of cases	No. of cases (%) with MS-1⁺ vessels	Vascularity*
Normal skin	6	0	+
Vascular lesions†	15	13	+/++++
Histiocytic lesions‡	11	36	+/++++
Melanocytic nevi	12	33	+
Primary melanoma	6	50	+
Melanoma metastasis	7	71	+++
Cutaneous T-cell lymphoma	5	80	++
Psoriasis	7	71	++
Granulation tissue	3	100	++++

* Number of 1F10⁺ vessels: +, normal; ++, slightly increased; +++, increased; ++++, highly increased.

† Naevus flammeus (port wine stain) with teleangiectasia (1), teleangiectasia (1), cavernous hemangioma (2), capillary hemangioma (1), cherry angioma (1), angiokeratoma (1), livedo reticularis (1), atypical M. Kimura (1), and AIDS Kaposi's sarcoma (6).

‡ Dermatofibrosarcoma protuberans (1), neurofibroma (1), malignant fibrous xanthoma/histiocytoma (1), cheilitis granulomatosa (1), histiocytoma (1), histiocytosis X (1), sarcoidosis (3), and juvenile xanthogranuloma (2).

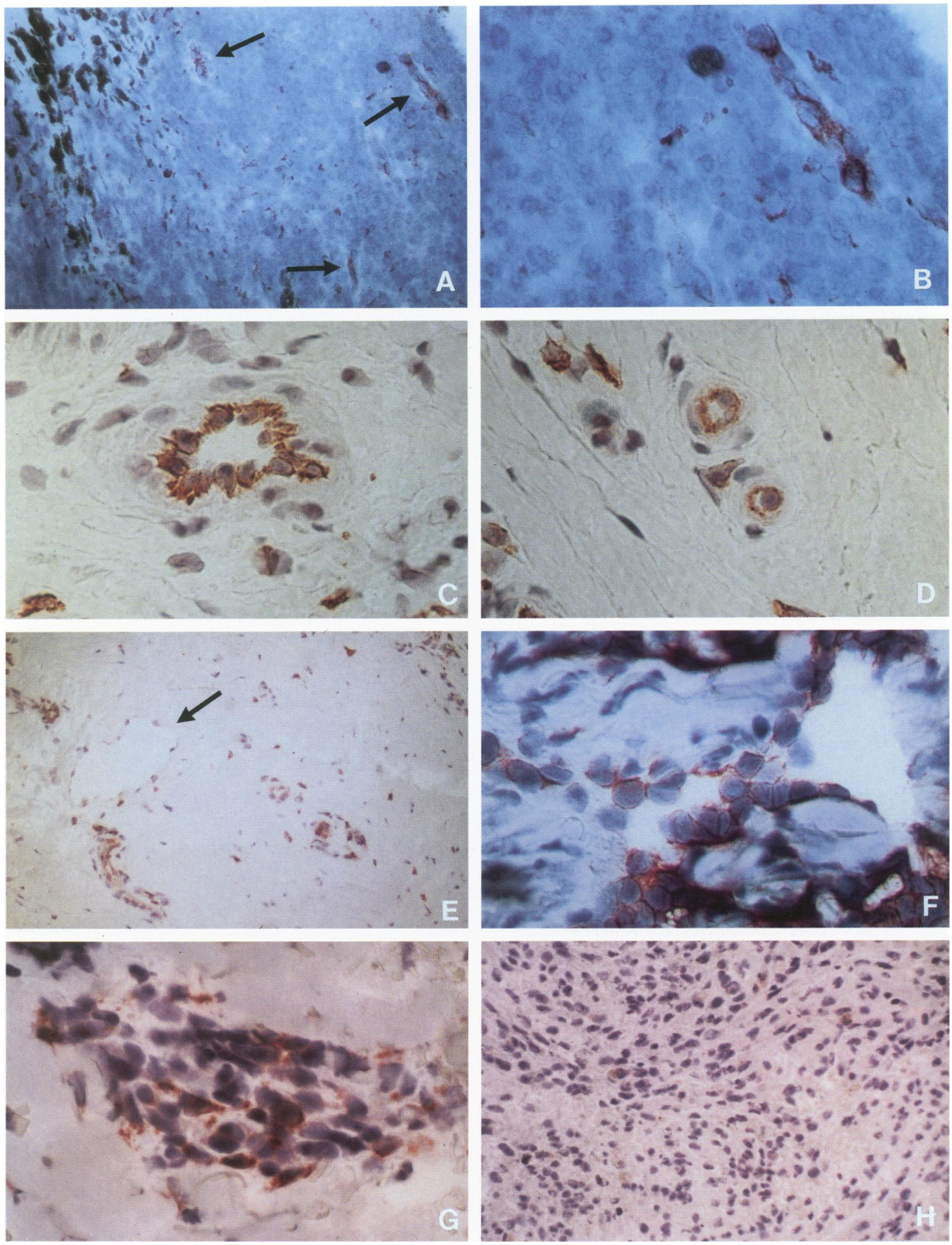

Figure 1. *Expression of MS-1 antigen by endothelial cells of continuous origin in skin lesions and in Kaposi's sarcoma. Immunohistochemical labeling with mAb MS-1 using immunoperoxidase (red reaction product) and hematoxylin (blue) as a counterstain.* **A**, *primary melanoma. Endothelial cells of small tumor vessels (collapsed veins; arrows) are moderately stained. × 140.* **B**, *higher magnification (× 225) of MS-1-positive vessel in* **A**. **C–E**, *wound healing. MS-1 expression is strong in endothelial cells of small arteries* (**C**; *× 225*) *and arterioles* (**D**; *× 225*), *while it is weak in or may even be absent from lymphatic endothelia in the same lesion* (**E**; *arrow; × 140*). **F**, *port wine stain with teleangiectasia. The endothelial lining of a single highly contorted and dilated vein shows MS-1 antigen expression. × 225.* **G**, *early patch lesion of AIDS Kaposi's sarcoma. A large number of cells express MS-1 antigen. × 225.* **H**, *spindle cell nodule of AIDS Kaposi's sarcoma. No MS-1 staining in the neoplastic cells or in the tumor vasculature. × 140.*

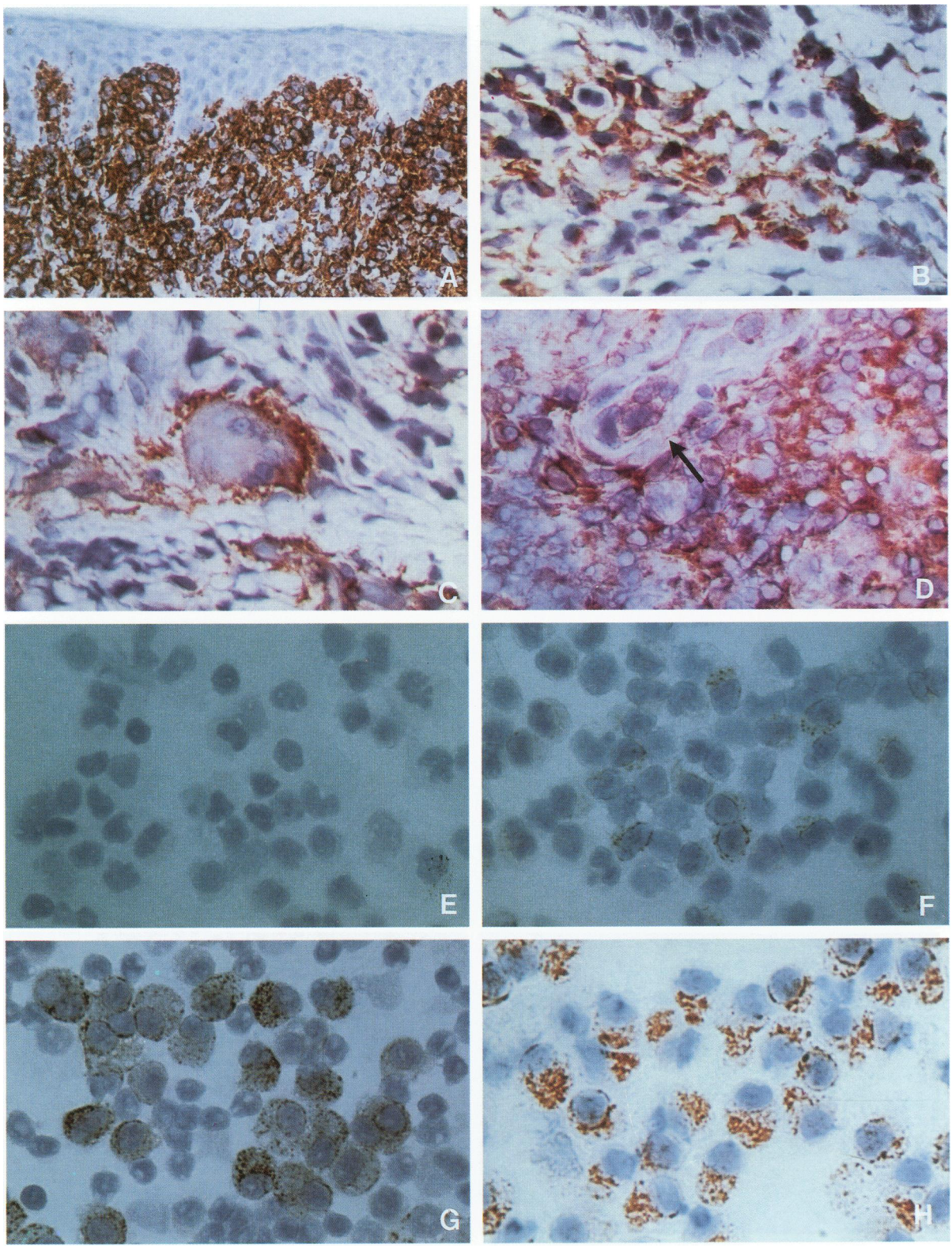

Figure 2. *Expression of MS-1 antigen by macrophages in skin lesions and in vitro. Immunochemical labeling with mAb MS-1 using immnuoperoxidase (red reaction product) and hematoxylin (blue) as a counterstain.* **A**, *early lesion of xanthogranuloma juvenile (nevoxanthoendothelioma). Numerous lesional macrophages strongly express MS-1 antigen.* × *140.* **B** *and* **C**, *late lesion of xanthogranuloma juvenile. Macrophages strongly expressing MS-1 antigen are still abundant but less numerous than in the early stage* (**B**; × *225*). *Numerous giant cells can be seen with a ring of MS-1-positive material at the cell periphery and a cytoplasm almost devoid of MS-1 antigen* (**C**; × *225*). **D**, *M. Kimura developing into malignant*

Results

MS-1 Antigen Expression in Endothelial Cells of Continuous Origin

In the course of our initial histological survey with mAb MS-1, we found that endothelial MS-1 antigen expression is not absolutely restricted to noncontinuous sinusoidal endothelia but that endothelial cells of apparently continuous origin in a subpopulation of vessels in tonsil[30] and in 8 of 10 carcinomas (especially rectal and lung adenocarcinomas; S. Goerdt and J. S. Pober, unpublished observations) also expressed MS-1 antigen, although the intensity of endothelial MS-1 antigen expression was relatively low. This prompted us to more closely investigate endothelial MS-1 antigen expression in a broad range of different skin lesions (in skin all blood vascular endothelia are of continuous origin) using mAb 1F10 (continuous type endothelia) and RM-3/1 (monocytes and tissue macrophages) as controls. In normal skin, we did not find MS-1-positive vessels, while there were cases with MS-1-positive vessels in all groups of pathological specimens. The percentage of cases with MS-1-positive vessels was high in granulation tissue/wound healing (100% of cases with vascular MS-1 expression), cutaneous T-cell lymphoma (80%), psoriasis (71%), and melanoma metastases (71%) and was intermediate in primary melanomas (50%) (Table 1). In a given MS-1-positive vessel, all endothelial cells regularly expressed MS-1 reactivity. Staining of serial sections by both mAb MS-1 and mAb 1F10 and double labeling of selected cases (including the wound healing specimens) with both mAbs showed that MS-1-positive vessels that could be identified as such by light microscopic demonstration of a lumen regularly expressed both MS-1 and 1F10 antigens. In general, the relative number of MS-1-positive vessels in a given lesion was low (<5% of the number of 1F10 positive vessels), and the MS-1 staining was less intense than that of lesional dendritic macrophages (Figure 1**A** and 1**B**); only in wound healing (Figure 1**C–E**) (and in full-fledged plaque or tumor stage cutaneous T-cell lymphoma) was a higher percentage of 1F10-positive vessels MS-1 positive, and endothelial MS-1 expression was comparatively strong here. In wound healing tissue, endothelial MS-1 expression was especially prominent in small arteries (Figure 1**C**) and arterioles (Figure 1**D**), while in cutaneous T-cell lymphomas it was the venous endothelial cells that preferentially expressed MS-1 antigen. Lymphatic endothelia in these same lesions did not express significant amounts of MS-1 antigen (Figure 1**E**). MS-1-positive nondendritic single cells, which were especially numerous in wound healing lesions, were only partially double labeled by mAb 1F10. The percentage of cases with MS-1-positive endothelial cells was low in melanocytic and nevocellular nevi (33%) and in histiocytic (36%) and vascular (13%) lesions (Table 1). In the vascular tumors examined, only a highly ectatic, highly contorted vascular structure of a single port wine stain was lined by MS-1-reactive endothelial cells (Figure 1**F**). In general, the occurrence of MS-1-positive vessels was not proportional to the mean vascularity of a given lesion as judged by the total number of 1F10-positive vessels (Table 1). For example, juvenile xanthogranuloma and granulation tissue are both highly vascularized, but MS-1-positive vessels were found only in granulation tissue; and both cutaneous T-cell lymphoma and melanocytic nevi are only moderately vascularized, but MS-1-positive vessels were frequently found in cutaneous T-cell lymphoma. In addition, when all malignant lesions were grouped together and compared with the benign lesions, the percentage of cases with MS-1-positive vessels was only marginally higher in the malignant group (55% versus 31% of cases with vascular MS-1 expression); when melanocytic nevi, primary melanomas, and melanoma metastases were compared, however, an increase in the percentage of cases with MS-1-positive vessels was seen (31%, 50%, and 71%, respectively). Interestingly, in AIDS Kaposi's sarcoma, a tumorous lesion of supposed endothelial (either blood vascular or lymphatic) origin, expression of MS-1 antigen was not seen in 1F10-positive vessels; the spindle cells of late-stage Kaposi's lesions were MS-1-negative as well (Figure 1**H**). In contrast, a large number of dendritic cells in the early patch lesions of Kaposi's sarcoma showed MS-1 antigen expression (Figure 1**G**).

MS-1 Antigen Expression by Dendritic Cells/Macrophages

In situ, MS-1 antigen expression by MS-1 nonendothelial interstitial cells (dendritic cells/macrophages)

lymphoma. Numerous lesional cells express MS-1 antigen; endothelial cells of most lesional hyperplastic small veins are weakly MS-1 reactive (arrow). × 225. **E**, *cultured human monocytes/macrophages day 1. No MS-1-positive cells can be detected. × 225.* **F**, *cultured human monocytes/macrophages day 3. About 15% of cells are weakly MS-1-positive. × 225.* **G**, *cultured human monocytes/macrophages day 9. About 65% of cells (mostly large macrophages) express MS-1 antigen in a granular, cytoplasmic pattern. × 225.* **H**, *human monocytes/macrophages cultured for 3 days with dexamethasone (5×10^{-7} mol/L). About 95% of cells strongly express MS-1 antigen (compare with* **F**). *× 225.*

Table 2. *Expression Kinetics of MS-1 and RM-3/1 Antigens and CD23 in Cultured Human, Single Donor Monocytes/Macrophages*

Culture period (days)	Antigen expression (% ± SD)†		
	MS-1	RM-3/1	CD23
1	0	79 ± 14	1 ± 1
3	15 ± 7	89 ± 10	1 ± 2
6	44 ± 2	78 ± 12	2 ± 1
9	63 ± 6	31 ± 8	4 ± 5
12	24 ± 6	29 ± 6	0
15	22 ± 3	37 ± 26	0

† Mean values of three single donor experiments. Number of antigen positive cells as percentage ± SD.

was always quite strong, while the number of these MS-1-positive cells varied considerably. MS-1-positive macrophages were especially numerous in the case of a young lesion of juvenile xanthogranuloma (Figure 2**A**); a similar pattern was seen in the case of an atypical M. Kimura developing into malignant lymphoma (Figure 2**D**). In an older lesion of juvenile xanthogranuloma, MS-1-positive macrophages were less numerous (Figure 2**B**); the numerous giant cells in the lesion showed deposits of MS-1-reactive material at the cell periphery, while the cytoplasm of these cells did not contain MS-1 antigen (Figure 2**C**). MS-1 antigen expression in these lesions was compared with expression of CD23 and RM-3/1 antigen; RM3/1 antigen was expressed by all MS-1-positive cells but was also seen in a considerable portion of MS-1-negative macrophages; in contrast, expression of CD23 was not found in MS-1-positive macrophages.

The observation that dendritic macrophages *in situ*, but not monocytes, can express MS-1 antigen prompted us to investigate whether MS-1 antigen might be inducible in monocytes/macrophages in culture and to determine how its expression might be regulated in comparison to RM 3/1 antigen and CD23. MS-1 antigen can be expressed by human monocytes/macrophages in culture (Figure 2**E–H**); MS-1 antigen expression by human monocytes/macrophages is first observed in about 15% of cells after 3 days of culture (Figure 2**E** and 2**F**) in a granular, cytoplasmic pattern; the percentage of MS-1 antigen positive cultured monocytes/macrophages peaks after about 9 days of culture (63%) (Figure 2**G**) and rapidly declines thereafter (24% at day 12, 22% at day 15) (Table 2). When monocytes were cultured with various cytokines (IL-1β, IL-4, IL-6, IFN-γ, TNF-α, granulocyte-monocyte colony-stimulating factor, or monocyte colony-stimulating factor) or the glucocorticoid dexamethasone for 3 or 6 days (Tables 3 and 4, respectively), IFN-γ turned out to be an absolute inhibitor for MS-1 antigen expression and a strong inhibitor for RM 3/1 antigen expression; dexamethasone had the strongest stimulatory effects on MS-1 antigen expression (Figure 2**H**) and, as expected, on RM 3/1 antigen expression; IL-4 and monocyte colony-stimulating factor exerted weak stimulatory effects; and TNF-α exerted weak inhibitory effects on MS-1 and RM 3/1 antigen expression (IL-4 had a more pronounced stimulatory effect on RM 3/1 antigen when cell surface expression was measured by flow cytometry; not shown); while granulocyte-monocyte colony-stimulating factor, IL-1β, and IL-6 seemed to have no clear-cut effects in this respect. The strongest stimulatory effects on MS-1 antigen expression were observed when IL-4 and dexamethasone were used in combination. In contrast to MS-1 and RM 3/1 antigen expression, IL-4 and IFN-γ were confirmed to have strong and weak stimulatory effects on CD23 expression in cultured human monocytes/macrophages, respectively, while dexamethasone was inhibitory. Immunoprecipitation with mAb MS-1 from lysates of IL-4- and dexamethasone-treated *versus* IFN-γ-treated monocytes/macrophages revealed that MS-1 protein, as expected, is expressed only in MS-1 antigen positive, IL-4/dexamethasone-treated cells and is absent from IFN-γ-treated cells and A375 melanoma cells (Figure 3). Immunoprecipitation with mAb MS-1 from lysates of IL-4- and dexamethasone-treated monocytes/macrophages and from spleen lysates showed that the pattern of protein bands is similar; the molecular weight of the macrophage protein species is slightly smaller than that of the respective spleen-derived bands.

The action of dexamethasone, the strongest stimulus for MS-1 antigen expression in cultured human monocytes/macrophages, was more closely examined in a separate time course experiment (Table 5). Dexamethasone was added to human monocytes/macrophages either at the onset of the culture period or 1 day before harvest; antigen expression was analyzed at days 0, 1 ,3 , 5, and 7. Under conditions of continuous treatment, the onset of dexamethasone action on MS-1 antigen expression is rather delayed (little enhancement on day 1, peak expression day 5); in comparison, when dexamethasone treatment was carried out for only 1 day before analysis, the onset of its action was similarly delayed, but the effectiveness of the treatment was reduced (about 20% fewer MS-1-reactive cells at all time points); this suggests that dexamethasone may induce a state of macrophage differentiation, permissive for MS-1 antigen expression, rather than directly alter MS-1 transcription/translation processes. In addition, the dependence of the modulatory po-

Table 3. *Expression of MS-1 and RM 3/1 Antigens and CD23 by Human Monocytes/Macrophages Cultured with Various Cytokines for 3 Days*

Cytokine treatment*	Antigen expression (% ± SD)†		
	MS-1	RM 3/1	CD23
—	53 ± 30	61 ± 25	2 ± 1
IFN-γ	0	22 ± 9	16 ± 10
TNF-α	28 ± 13	61 ± 10	2 ± 1
IL-6	33 ± 33	62 ± 20	15 ± 15
IL-1β	47 ± 29	64 ± 11	14 ± 5
GM-CSF	61 ± 7	73 ± 4	27 ± 29
M-CSF	76 ± 32	93 ± 3	6 ± 6
IL-4	77 ± 4	68 ± 13	46 ± 8
Dexamethasone	94 ± 6	100	0
Dexamethasone + IL-4	99 ± 2	100	10 ± 11

* Addition of cytokines or dexamethasone at the onset of a 3-day culture period.
† Mean values of 3 separate experiments. Number of antigen positive cells as percentage ± SD.
Abbreviations: GM-CSF, granulocyte-monocyte colony-stimulating factor; M-CSF, monocyte colony-stimulating factor.

tential of IFN-γ on its concentration and on combinations with other stimuli was thoroughly analyzed (Table 6). As for inhibition of MS-1 and RM 3/1 antigen expression, IFN-γ is equally effective in control and IL-4-stimulated cultured human monocytes/macrophages, and the inhibitory action is already measurable at a concentration of 10 U/ml of IFN-γ. In dexamethasone-treated monocytes/macrophages, the inhibitory effect of IFN-γ is less pronounced: a slight inhibitory effect is seen only at a concentration of 100 U/ml of IFN-γ, and the effect at 1000 U/ml is in the range of only 50% MS-1 antigen positive cells compared to 1% in the control population; in IL-4- and dexamethasone-treated monocytes/macrophages, there is just a minimal inhibitory effect on MS-1 antigen expression at 1000 U/ml of IFN-γ (71% MS-1 antigen positive cells). In comparison, there is no inhibitory effect of IFN-γ on RM 3/1 antigen expression in dexamethasone- or in dexamethasone- and IL-4-treated monocytes/macrophages. In contrast to MS-1 and RM 3/1 antigens, CD23 is tremendously up-regulated by combined treatment with IL-4 and IFN-γ, while dexamethasone thoroughly suppresses CD23 expression in IL-4- and/or IFN-γ-treated monocytes/macrophages, except when IL-4 is combined with IFN-γ at the highest concentration (1000 U/ml).

Discussion

We report here that MS-1 high-molecular-weight protein antigen[30,31] is expressed in a proportion of vascular endothelia of continuous origin in pathological skin specimens. However, we have not been able to establish the exact pathobiological conditions nor the inductive mechanisms for MS-1 antigen expression by endothelia of continuous origin. Overall vascularity of a given lesion or malignancy are clearly not correlated with MS-1 induction in continuous endothelia. Increased vascularity has been shown to be indicative of tumor progression in selected examples.[51] In this setting, increased vascularity may be taken as a token of increased angiogenic activity of the developing malignant lesions. We suggest that increased endothelial MS-1

Table 4. *Expression of MS-1 and RM 3/1 Antigens and CD23 by Human Monocytes/Macrophages Cultured with Various Cytokines for 6 Days*

Cytokine treatment*	Antigen expression (% ± SD)†		
	MS-1	RM 3/1	CD23
—	53 ± 3	61 ± 15	5 ± 10
IFN-γ	0	10 ± 8	15 ± 13
TNF-α	21 ± 6	39 ± 7	3 ± 1
IL-6	50 ± 14	60 ± 10	5 ± 4
IL-1β	49 ± 10	63 ± 11	10 ± 8
GM-CSF	52 ± 5	50 ± 13	27 ± 17
M-CSF	63 ± 19	78 ± 22	6 ± 7
IL-4	61 ± 3	56 ± 14	45 ± 30
Dexamethasone	93 ± 6	99 ± 1	1 ± 0
Dexamethasone + IL-4	100	100	20 ± 26

* Addition of cytokines or dexamethasone at the onset of a 3-day culture period.
† Mean values of 3 separate experiments. Number of antigen positive cells as percentage ± SD.
Abbreviations: GM-CSF, granulocyte-monocyte colony-stimulating factor; M-CSF, monocyte colony-stimulating factor.

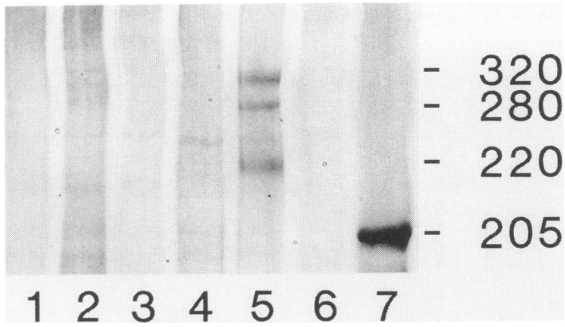

Figure 3. *Comparison of antigen immunoprecipitated from A375 melanoma cell lysates (lanes 1 and 2) and from lysates of human monocytes/macrophages cultured for 3 days with 1000 U/ml of IFN-γ (lanes 3 and 4) or with dexamethasone (5×10^{-7} mol/L) and 300 U/ml of IL-4 (lanes 5 and 6) by mAb MS-1 (lanes 2, 4, and 5), with isotype-matched mAb as a specificity control (lanes 1, 3, and 6); high-molecular-weight standard (myosin) was run in lane 7. Samples were run on a 5% polyacrylamide gel after reduction. The gel front was allowed to migrate off the bottom of the gel to achieve optimal resolution in the high-molecular-weight range. Proteins were visualized by silver staining. MAb MS-1 precipitates 320, 280, and 220 kd protein species from IL-4- and dexamethasone-treated cultured human monocytes/macrophages but not from IFN-γ-treated cultured human monocytes/macrophages or from A375 melanoma cells.*

expression in melanoma metastasis compared to primary melanoma and melanocytic nevi may be interpreted as relating to this phenomenon. In general, overall vascularity is only a good measure for past angiogenesis but does not reflect the actual angiogenic potential of a given lesion at a certain time point since it is cumulative and cannot account for transient processes. Unfortunately, there is as yet no direct independent measure for the angiogenic activity of lesions in tissue sections, ie, there is no monoclonal antibody that selectively stains endothelial cells undergoing angiogenesis. Thus, the question of whether MS-1 antigen expression by endothelial cells of continuous origin might be involved in angiogenic processes cannot be answered experimentally; the frequent occurrence of MS-1 positive vessels in moderately vascularized cutaneous T-cell lymphoma, however, argues against this hypothesis. MS-1-positive vessels that can be identified as such by light microscopic demonstration of a lumen regularly expressed both MS-1 antigen and 1F10 (continuous type endothelial cell) antigen. Positivity for both of these antigens demonstrates that MS-1-positive endothelial cells in skin lesions not only are of continuous origin but retain characteristics of continuous type endothelia. MS-1 protein is not the only antigen that has been reported to be inducibly expressed by endothelial cells of continuous origin under comparable conditions. Syndecan, an epithelium-associated cell surface proteoglycan with multiple functions in cell/matrix and cell/cell adhesion, in growth factor binding, morphogenesis, and tissue regeneration, has just recently been demonstrated to be transiently expressed (days 3 and 4) by endothelial cells of selected capillaries in the granulation tissue of an unsutured mouse wound healing model.[52] Therefore, we offer the hypothesis that continuous type endothelia may express *de novo* special molecules such as MS-1 and syndecan under certain pathological conditions to preserve their integrity.

In addition, we report here that MS-1 antigen is expressed not only by interstitial dendritic cells in certain normal human organs such as placenta[30] and skin,[31] but that MS-1-positive dendritic cells/macrophages constitute a natural part of most of the pathological specimens examined. The number of MS-1 antigen positive lesional dendritic macrophages varies in an unpredictable manner in most lesions, even within a given disease entity. In contrast, in AIDS Kaposi's sarcoma a high number of

Table 5. *Expression Kinetics of MS-1 and RM 3/1 Antigens and CD23 in Cultured Human Monocytes/Macrophages Treated with Glucocorticoid*

Culture*/treatment† duration (days)	Antigen expression (% ± SD)‡		
	MS-1	RM 3/1	CD23
0/—	1 ± 1	72 ± 22	1 ± 1
1/—	1 ± 2	83 ± 11	1 ± 2
1/1	14 ± 27	98 ± 1	0
3/—	41 ± 40	88 ± 13	1 ± 2
3/1	67 ± 27	98 ± 0	1 ± 1
3/3	83 ± 22	100	0
5/—	56 ± 49	73 ± 9	2 ± 2
5/1	79 ± 11	82 ± 7	3 ± 2
5/5	94 ± 7	99 ± 1	0
7/—	35 ± 50	34 ± 4	0
7/1	53 ± 12	57 ± 2	0
7/7	91 ± 15	99 ± 1	0

* Duration of culture after isolation from 1-day-old buffy coats.
† Duration of dexamethasone treatment (5×10^{-7} mol/L): no treatment (—), addition 1 day before harvest (1), addition at the onset of the culture period (3, 5, 7).
‡ Mean values of 3 separate experiments. Number of antigen positive cells as percentage ± SD.

Table 6. *Concentration-Dependent Inhibition/Induction by γ-Interferon of MS-1 and RM 3/1 Antigen and CD23 Expression in Interleukin-4- and/or Dexamethasone-Treated Cultured Human Monocytes/Macrophages*

Treatment*	Antigen expression (%)†		
	MS-1	RM 3/1	CD23
Control	62	73	0
IFN-γ 10	44	64	1
IFN-γ 100	0	34	33
IFN-γ 1000	1	21	29
IL-4	90	65	80
IL-4 + IFN-γ 10	79	66	52
IL-4 + IFN-γ 100	20	40	74
IL-4 + IFN-γ 1000	3	15	91
Dexamethasone	97	100	1
Dexamethasone + IFN-γ 10	98	99	0
Dexamethasone + IFN-γ 100	85	98	0
Dexamethasone + IFN-γ 1000	54	96	3
IL-4 + dexamethasone	100	100	3
IL-4 + dexamethasone + IFN-γ 10	100	100	3
IL-4 + dexamethasone + IFN-γ 100	98	99	2
IL-4 + dexamethasone + IFN-γ 1000	71	100	63

* Substances were added together at the onset of a culture period of 3 days. Concentrations were 300 U/ml IL-4, 5×10^{-7} mol/L dexamethasone, and 10, 100, and 1000 U/ml γ-IFN.

† Data of a representative experiment. Number of antigen expressing cells as a percentage.

MS-1 antigen positive interstitial dendritic cells are found in the early patch lesions. This is in good concordance with the high number in AIDS Kaposi's sarcoma of factor XIIIa+ interstitial dendritic cells,[11,24] of which MS-1 antigen positive dendritic cells seem to constitute a subpopulation.[31] In juvenile xanthogranuloma, the second lesion with extraodinarily high numbers of MS-1-positive macrophages, MS-1 expression seems to be tightly regulated during development from the early to the mature xanthomatous form. Early lesional histiocytes show intense cytoplasmic staining, while aging foamy and multinucleated giant cells exhibit MS-1 positivity as a thin band at the cell periphery. Noticeably, both IL-4 and IFN-γ are known to induce giant cell formation in cultured monocytes/macrophages.[53–55]

In vitro, MS-1 antigen is an intermediate to late differentiation antigen of human monocytes/macrophages. Its expression in these cells is strongly enhanced by the action of the glucocorticoid, dexamethasone, and by IL-4, while it is inhibited by IFN-γ and TNF-α. RM 3/1 antigen,[48,49] preferentially expressed by macrophages in the healing phase of inflammation, is regulated in a similar fashion. In contrast, cytoplasmic CD23 expression in macrophages is stimulated by IL-4[56,57] and IFN-γ (IFN-γ in addition diminishes cell surface expression and enhances the release of CD23),[58,59] while it is inhibited by glucocorticoid.[60] Corticosteroids, beyond their clinically anti-inflammatory and immunosuppressive actions, are known to mostly exert suppressive effects on various inflammatory macrophage functions, such as elastase, collagenase, and plasminogen activator secretion;[61] IL-1,[62] IL-6,[63] TNF,[64] and prostaglandin E_2 production;[65] and antimicrobial activity.[66] In addition, Poulter et al have proposed a concept of (corticosteroid-inducible) suppressor macrophages by identifying a macrophage phenotype (RFD7 antigen positive) which suppresses the allogeneic mixed lymphocyte reaction and is induced by corticosteroid *in vitro*.[67–70] Furthermore, it is well known that IL-4 shares and augments certain anti-inflammatory glucocorticoid actions on macrophages such as suppression of IL-1, IL-6, TNF-α, and prostaglandin E_2 production;[63,65] corticosteroids, on the other hand, induce enhanced secretion of IL-4 by T-cells *in vivo* and *in vitro*.[71] Thus, when IL-4 is combined with corticosteroids its anti-inflammatory effects seem to prevail. MS-1 antigen expression by macrophages *in vitro* clearly follows an anti-inflammatory pattern of agent action (dexamethasone, IL-4, and dexamethasone and IL-4 enhance MS-1 expression; IFN-γ can only suppress MS-1 expression when used alone or in combination with IL-4; when combined with dexamethasone, IFN-γ is much less effective).

In conclusion, we have shown here that MS-1 high-molecular-weight protein is tightly regulated *in vivo* and *in vitro* and is involved in the pathobiology of human endothelium and dendritic cells/macrophages.

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