American Journal of Pathology, Vol. 155, No. 4, October 1999

Animal Model

Expression of E-Selectin, P-Selectin, and Intercellular Adhesion Molecule-1 during Experimental Murine Listeriosis

Santiago López, Neus Prats, and Alberto Jesús Marco

From the Department of Pathology and Animal Productions, Veterinary School, Bellaterra, Barcelona, Spain

The expression of adhesion molecules E-selectin, P-selectin, and intercellular adhesion molecule-1 (ICAM-1) was immunohistochemically investigated during the course of experimental murine listeriosis. Infection was monitored by microbiological count of blood, liver, and spleen. After an early generalized expression of P-selectin and ICAM-1, a later regulation occurred specifically to areas of inflammation. Expression of E-selectin was faint and inconstantly detected in all of the studied organs. In the liver, typical lesions of murine listeriosis were related to the expression of ICAM-1 on sinusoidal endothelial cells and the biliary system and to the *de novo* expression of P-selectin in hepatic portal vessels. Inflammation in the spleen was related to the expression of ICAM-1 on red pulp sinusoidal cells, especially in the marginal sinus. High endothelial venules of inflamed lymph nodes also expressed P-selectin and ICAM-1. Lesions in the central nervous system appeared on day 3 after infection as a pyogranulomatous leptomeningitis associated with an intense expression of P-selectin and ICAM-1 in meningeal vessels, especially those in the hippocampal sulcus, suggesting a way through which inflammation initially reach the central nervous system during experimental murine listeriosis. Leptomeningitis was followed by the presence of ventriculitis, which was related to the up-regulation of ICAM-1 on choroid plexus epithelial cells, periventricular vessels and ependymal cells. Up-regulation of P-selectin and ICAM-1 during experimental murine listeriosis could play an important role in the recruitment of leukocytes, especially to the liver, lymphoid organs, and central nervous system. *(Am J Pathol 1999, 155:1391–1397)*

Experimental murine listeriosis has long served as a model for studying host defense against infections caused by intracellular pathogens in general,[1] and listeriosis in particular.[2–6]

Systemic murine listeriosis is characterized by the rapid influx of leukocytes, especially neutrophils but also macrophages, into the site of initial bacterial replication, especially the liver and spleen. These cells have been shown to be essential for the early defense against the infection.[7,8] Leukocyte recruitment to inflammatory sites consists of a complex series of interactions mediated by cell adhesion molecules expressed on the surface of inflammatory and endothelial cells. The initial step in the adhesion cascade is the tethering and rolling of leukocytes along the endothelium, which is mediated by the interaction of members of the selectin family and their carbohydrate ligands.[9–11] E- and P-selectin, expressed on the endothelial cell surface, have been shown to support the initial rolling phase of neutrophils and monocytes *in vitro* and *in vivo*. Firm adhesion to endothelium and subsequent emigration through the vessel wall is dependent on leukocyte $\beta 1$ and $\beta 2$ integrin activation and their interaction with members of the immunoglobulin-like superfamily on the endothelium, including intercellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1).[9,11]

The use of the subcutaneous route of infection has been shown as a suitable model of systemic murine listeriosis, and can also reproduce central nervous system (CNS) lesions similar to those of listeria meningitis in humans and other species. The main feature of these lesions is the recruitment of inflammatory cells, especially macrophages and neutrophils, to the subarachnoid and ventricular space causing meningitis and choroiditis.[6]

Supported by the Comisión Interdepartamental de Ciencia y Tecnología (CICYT), AGF93-C02–02.

Accepted for publication June 15, 1999.

Address reprint requests to Santiago López, Histologia i Anatomia Patològica, Departament de Patologia i Produccions Animals, Facultat de Veterinària, Universitat Autònoma de Barcelona, 08193 Bellaterra, Barcelona, Spain. E-mail: santiago.lopez@cc.uab.es.

Despite of being extensively used as an experimental model for the study of the pathogenesis of and host defense against intracellular bacteria, the expression and contribution of endothelial cell adhesion molecules to the pathogenesis of *Listeria monocytogenes* during experimental murine listeriosis have not been characterized yet. Therefore, the aim of this study was to detect by immunohistochemistry the expression of E-selectin, P-selectin, and ICAM-1, especially in the liver, lymphoid organs and CNS of mice during the course of experimental murine listeriosis using the subcutaneous route of infection, and to determine whether correlation exists between the expression of these adhesion molecules and the inflammatory infiltrate.

Materials and Methods

L. monocytogenes (serovar 4b, strain P-14B) was grown on brain heart infusion broth (BHI, Difco) at 37°C for 24 hours with orbital shaking. After centrifugation the organisms were collected and resuspended in sterile 0.9% saline to the required dose.

Animals

Forty-eight female 25-g SPF CD1 mice (Interfauna, Spain) were inoculated subcutaneously with 5×10^8 colony-forming units of viable *L. monocytogenes* in a 0.2 ml of solution in the lumbar zone. At various time intervals (days 1, 2, 3, 4, 5, 8, 11 after infection) (p.i.) groups of mice were killed by anesthetic (halothane) overdose. Immediately, blood collected from the cava vein was placed in heparin-coated microtubes (Becton Dickinson, Franklin Lakes, NJ) and 0.5 ml were directly plated on brain-heart infusion agar (BHIA). Samples of liver and spleen were aseptically removed, homogenized in Tris-buffered saline, and plated in 10-fold serial dilutions in BHIA for bacterial count. Plates were incubated for 24 hours at 37°C.

Samples of liver, spleen, lymph nodes, stomach, small and large intestine, pancreas, kidney, adrenal gland, urinary bladder, uterus, ovary, lung, heart, thymus, bone marrow, spinal cord, and brain were taken immediately after killing the animals, fixed for 48 hours in buffered formalin, embedded in paraffin, and processed for histopathology and immunohistochemistry.

Animal experiments were performed under the supervision of the Animal Care Committee of the Universitat Autònoma de Barcelona.

Immunohistochemistry

Primary rat monoclonal antibodies against mouse ICAM-1 (clone KAT-1) (R&D Systems, Abingdon, UK), E-selectin (clone 10E9C) (Pharmingen, San Diego, CA), neutrophils (clone 7/9) (Immunokontact, Bioggio, Switzerland) and polyclonal rabbit against mouse P-selectin (Pharmingen) were used. Immunohistochemistry to detect ICAM-1 and E-selectin was performed as previously described.[12] To detect P-selectin and neutrophils in formalin-fixed and paraffin-embedded tissues, sections were placed in 0.01 mol/L citrate buffer (pH 6) and heated for 10 minutes (P-selectin) or 5 minutes (neutrophils) in a microwave oven (Moulinex FM A735A, 850 W) for antigen retrieval. After blocking nonspecific binding, sections were incubated with the primary antibody diluted 1:250 (P-selectin) and 1:800 (neutrophils) in 0.05 mol/L Tris-buffered saline, pH 7.6, at 4°C overnight. A biotinylated goat anti-rabbit IgG and a biotinylated goat anti-rat IgG (Dako, Glostrup, Denmark) were used as a secondary antibodies diluted in Tris-buffered saline, pH 7.6, at 1:400 and 1:200, respectively. Reaction was developed with the avidin-biotin horseradish peroxidase complex using as chromogen 0.05% solution of 3,3′-diamenobenzidine (Sigma Chemical Co., St. Louis, MO) with 0.03% H_2O_2 in 0.1 mol/L imidazole buffer (pH 7.1). *L. monocytogenes* was immunohistochemically detected by a modified technique from Domingo et al[13] using the avidin-biotin complex system (Dako). Sections incubated with isotype-matched antibodies served as negative controls in each technique.

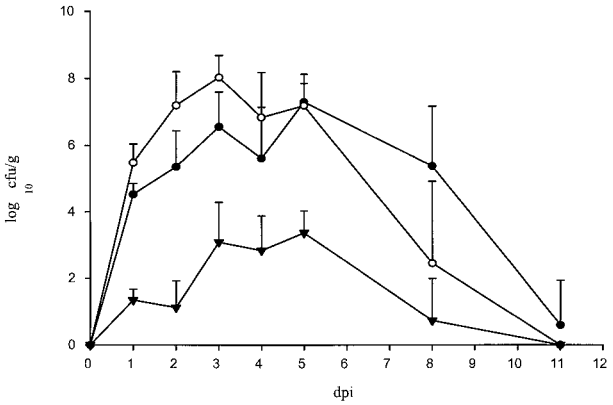

Figure 1. Bacterial growth in organs of mice infected subcutaneously with 5×10^8 colony-forming units of viable *L. monocytogenes* in liver (●), spleen (○), and blood (▼). Data are expressed as the mean ± SD (five to eight mice per time point).

Results

Clinical and Microbiological Results

Neither symptoms nor deaths were seen until day 4 p.i., with the exception of one mouse that died on day 3 p.i. On day 4 p.i. many animals rested immobilized and showed hair bristling. From day 5 p.i. to the end of the experiment, most animals showed neurological signs such as ataxia and tremors, and three of them, circling. During this period two animals died and many had to be euthanized due to severe symptomatology. Results of bacterial counts in the liver, spleen, and blood are shown in Figure 1. *Listeria* was detected from day 1 p.i. through the end of the experiment. The maximum bacterial count in the liver (10^7 colony-forming units/g) and the spleen (10^8 colony-forming units/g) occurred between days 3 and 5 p.i., after which they gradually decreased and became undetectable on day 11 p.i. *Listeria* was de-

tected in blood at low levels (10/ml) during the first two days but reached levels of 10^3/ml on day 3 p.i. that lasted until day 5 p.i., then declined until there were undetectable levels on day 11 p.i. This period of bacteremia was coincident with the period of maximal bacterial replication in the spleen and liver.

Histopathology and Immunohistochemistry

Control Animals

As previously reported[12,14] no E-selectin expression was immunohistochemically detected in endothelial cells in any of the studied organs from control animals. Although P-selectin is preformed and stored in Weibel-Palade bodies of endothelial cells, platelets, and megakaryocytes of unstimulated animals,[15] P-selectin was only detected in megakaryocytes and circulating platelets of control animals. ICAM-1 was constitutively expressed in all of the studied organs. In the liver, ICAM-1 was faintly expressed on sinusoidal endothelial cells, especially around the centrilobular vein and also in some branches of the portal vein and hepatic artery. In lymphoid organs, ICAM-1 expression was detected in some splenic red pulp sinusoidal endothelial cells and in the center of lymphoid follicles, as well as in the medullary and subcapsular sinuses of lymph nodes. In the CNS, ICAM-1 was expressed in subarachnoid venules and capillaries, especially those in the hippocampal sulcus, and with less intensity in periventricular vessels. Choroid epithelial cells also expressed ICAM-1 in their apical membrane. ICAM-1 was also detected in venules and capillaries of the rest of organs, as well as in the endocardium and pulmonary alveolar epithelial cells.

Infected Animals

Typical lesions of septicemic listeriosis affecting the liver, spleen, and lymph nodes[1,3–5] began to appear on day 2 p.i. and were present until the end of the experiment. However, up-regulation and *de novo* expression of some of the adhesion molecules was already evident on day 1 p.i.

After inoculation, progressive ICAM-1 up-regulation in liver sinusoidal endothelial cells was observed, and reached the highest level of expression on days 2 to 3 p.i. and returned to basal levels on day 11 p.i. This up-regulation consisted not only of an increment in the intensity of the reaction but also of the distribution, which was markedly centrilobular on day 1 p.i. and became generalized during days 2 to 3 p.i. At this time, small pyogranulomatous foci of inflammation were randomly distributed throughout the hepatic parenchyma with the presence of *Listeria* in the center of the lesions. Sinusoidal endothelial cells immediately adjacent to these areas showed a more intense ICAM-1 staining (Figure 2A). This overexpression around the inflamed areas lasted to the end of the experiment, independently of the inflammatory cell composition, and the expression level in the rest of the sinusoidal cells. On days 3 to 4 p.i., lesions were pyogranulomatous and necrotizing with great numbers of *Listeria* and were most severe on day 5 p.i. On day 3 p.i., at the same time that sinusoidal expression was returning to its basal levels, a marked progressive up-regulation was detected in portal vessels associated with the presence of margination, diapedesis and periportal infiltration of neutrophils and macrophages. Moreover, ICAM-1 was *de novo* expressed in the latero-apical cell surface of biliary epithelial cells (Figure 2B). This expression in the portal area was maximum on day 5 p.i.; at this time inflammation and bacterial burden was maximum in this organ. Between days 5 and 8 p.i., pyogranulomatous cholecystitis and the presence of free and phagocytosed *Listeria* in bile ducts were common findings. Expression of P-selectin in the liver could be detected in centrilobular and portal veins and some portal capillaries on day 1 p.i. and became slightly reduced between days 2 and 3 p.i. However, on days 5 and 8 p.i. most of the animals had again marked expression of P-selectin in the centrilobular and portal veins and also in capillaries located near or within inflamed areas (Figure 2C). Between days 4 and 8 p.i. P-selectin was markedly expressed in the cytoplasm of circulating megakaryocytes in hepatic sinusoids. From day 8 p.i. to the end of the experiment, lesions became smaller and populated by a great proportion of macrophages and lymphocytes. On day 11 p.i. inflammation was almost absent, hepatocytes were vacuolated, and there was extramedullary hematopoietic activity with numerous megakaryocytes circulating within sinusoids and showing less P-selectin expression. Endothelial P-selectin expression had disappeared and ICAM-1 was at its basal levels with the exception of sinusoidal cells adjacent to small granulomas. E-selectin was only detected in some capillary and venule endothelial cells located in inflamed portal areas of the liver of two animals on days 3 and 4 p.i.

In the spleen, on day 2 p.i., inflammatory cells, predominantly neutrophils and macrophages, were mainly located in the periarteriolar lymphoid sheath and in red pulp, with higher presence in the marginal sinus. The inflammatory lesion was usually accompanied by a variable degree of lymphoid depletion in the periarteriolar lymphoid sheath. Between days 3 and 5 p.i. some animals showed a necrotizing splenitis. In these mice, lymphoid depletion was almost complete. From day 5 p.i. to the end of the experiment, lesions became more granulomatous, and the lymphocyte population was restored on day 11 p.i. Different degrees of *L. monocytogenes* immunostaining were always observed in macrophages and neutrophils associated with the lesions from day 2 to 8 p.i.

Neither P-selectin nor E-selectin was immunohistochemically detected in splenic endothelial cells from infected animals. Only megakaryocytes and platelets in the red pulp showed cytoplasmic P-selectin expression. On day 1 p.i., there was an up-regulation of the ICAM-1 constitutive expression in the spleen, and some animals also showed positive reaction in some nodular and trabecular arteries. Intralesional macrophages and those within the red pulp also showed ICAM-1 expression in the cell membrane. Endothelial expression in the red pulp

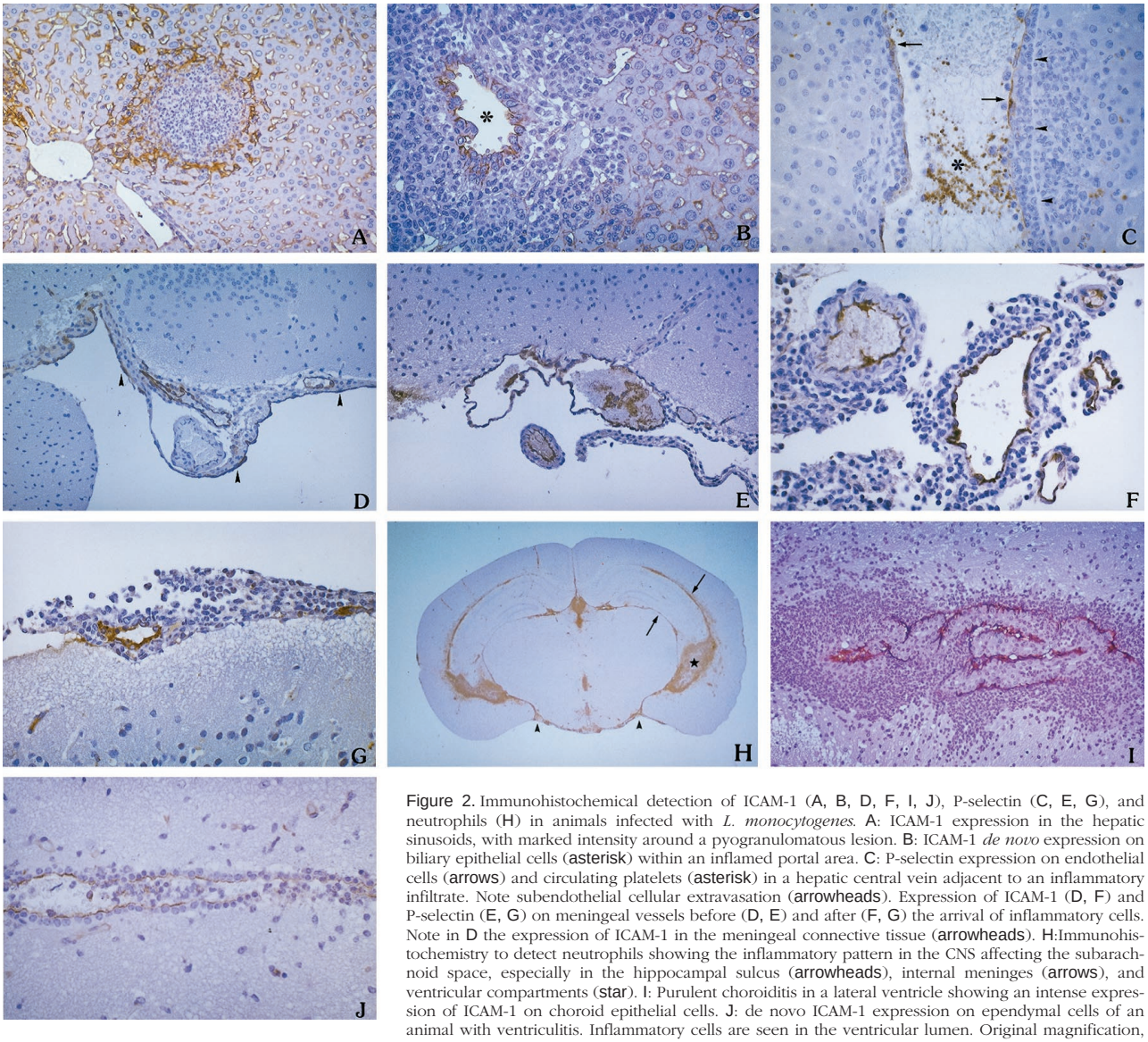

Figure 2. Immunohistochemical detection of ICAM-1 (A, B, D, F, I, J), P-selectin (C, E, G), and neutrophils (H) in animals infected with *L. monocytogenes*. A: ICAM-1 expression in the hepatic sinusoids, with marked intensity around a pyogranulomatous lesion. B: ICAM-1 *de novo* expression on biliary epithelial cells (asterisk) within an inflamed portal area. C: P-selectin expression on endothelial cells (arrows) and circulating platelets (asterisk) in a hepatic central vein adjacent to an inflammatory infiltrate. Note subendothelial cellular extravasation (arrowheads). Expression of ICAM-1 (D, F) and P-selectin (E, G) on meningeal vessels before (D, E) and after (F, G) the arrival of inflammatory cells. Note in D the expression of ICAM-1 in the meningeal connective tissue (arrowheads). H:Immunohistochemistry to detect neutrophils showing the inflammatory pattern in the CNS affecting the subarachnoid space, especially in the hippocampal sulcus (arrowheads), internal meninges (arrows), and ventricular compartments (star). I: Purulent choroiditis in a lateral ventricle showing an intense expression of ICAM-1 on choroid epithelial cells. J: de novo ICAM-1 expression on ependymal cells of an animal with ventriculitis. Inflammatory cells are seen in the ventricular lumen. Original magnification, ×214 (A, D, E, I); ×428 (B, C, F, G, J); ×128 (H). Hematoxylin counterstain.

was maximal on day 5 p.i., and returned to basal levels on day 11 p.i.

Lymph node lesions consisted of paracortical pyogranulomatous infiltrates associated with bacterial antigen. Although some animals had lesions in the inguinal and lumbar lymph nodes on day 1 p.i., most were present from day 2 p.i. through day 8 p.i. P-selectin and ICAM-1 but not E-selectin were *de novo* expressed on the endothelium of some veins in the medullary cords and high endothelial venules of some animals with lesions. Circulating macrophages in the medullary sinuses also showed intense ICAM-1 expression in their membrane.

Table 1. Time Course of Lesions in the CNS of Mice after Subcutaneous Inoculation of 5×10^8 Colony-Forming Units of Viable *L. monocytogenes*

Lesion	Days after Infection						
	1	2	3	4	5	8	11
Meningitis	0/6	0/8	6/8	6/8	7/8	5/5	2/5
Ventriculitis	0/6	0/8	0/8	1/8	5/8	2/5	1/5
Encephalitis	0/6	0/8	0/8	0/8	2/8	5/5	3/5

Data are number of affected animals per total.

CNS lesions appeared on day 3 p.i. and were present through experiment. The course of lesions is summarized in Table 1. As in the liver and spleen, induction and up-regulation of the studied adhesion molecules were detected soon after infection. ICAM-1 was already up-regulated on day 1 p.i., especially in subarachnoid venules and capillaries (Figure 2D), and was also expressed in large veins and arterioles. Subarachnoid vessels also showed *de novo* P-selectin expression (Figure 2E), which was more intense in the vessels of the hippocampal sulcus. Meningeal connective tissue, especially in this sulcus, also expressed ICAM-1 (Figure 2D). Some parenchymal vessels, and sinusoidal endothelial cells in the choroid plexus also had faint expression of P-selectin.

This expression pattern remained constant during the experiment but with less intensity, and only vessels adjacent to inflammation, showed intense ICAM-1 and P-selectin expression from day 3 p.i. through the end of the experiment (Figure 2, F and G). Lesions first appeared on day 3 p.i. and consisted of the presence of few inflammatory cells, predominantly neutrophils in the subarachnoid space. This inflammatory infiltrate was always more prominent in the hippocampal sulcus and hippocampus-associated internal meninges (Figure 2H). Presence of *L. monocytogenes* was associated with these lesions. At this time, a marked overexpression of P-selectin and ICAM-1 (Figure 2, G and F) was seen in meningeal venules and capillaries adjacent to the lesions, as well as, in the case of ICAM-1, in the meningeal connective tissue. On day 4 p.i., macrophages expressing ICAM-1 on the cell membrane were prominent in the meningeal lesions and some cells were in the ventricular lumen causing ventriculitis. On day 5 p.i. expression of ICAM-1 in meninges was maximal and all of the animals showed an intense pyogranulomatous meningitis with numerous macrophages, and most of them also suppurative ventriculitis with *Listeria*-laden neutrophils. In most of these animals, ventriculitis was associated with choroiditis and ependymitis, with necrosis of choroidal and ependymal cells. Animals with ventriculitis-choroiditis also showed increased expression of ICAM-1 on the choroid epithelial cells (Figure 2I) and, most, *de novo* expression on ependymal cells (Figure 2J). *Listeria*-laden neutrophils were detected adhered to ICAM-1-expressing ependymal cells (Figure 2J). In animals with ventriculitis, expression of ICAM-1 but not P-selectin, in periventricular vessels, especially venules, was clearly up-regulated in association with adhesion and diapedesis of neutrophils and macrophages. Some animals, from days 8 to 11 p.i., also showed perivascular accumulation of macrophages and neutrophils in the rombencephalum, with little *Listeria* antigen. These vessels showed intense expression of ICAM-1 while the rest of parenchymal vessels were faintly positive. P-selectin expression was not detected in these inflammatory lesions but some meningeal and parenchymal vessels, apparently unrelated to the infiltrate, had a slightly positive expression. On day 11 p.i. vascular and epithelial expression of ICAM-1 had returned to basal levels and only faint meningeal expression of ICAM-1 was still present.

As in other organs, E-selectin expression in the CNS was detected in few animals (4/47) and few vessels. It was expressed in some meningeal venules and capillaries in the subarachnoid space of the hippocampal sulcus. On day 5 p.i., expression in periventricular capillaries and in sinusoidal endothelial cells of the choroid plexus was detected in two animals.

In other organs, consistent lesions were multifocal pyogranulomatous adrenalitis (40%) and myocarditis (37%). These lesions were most severe and affected most animals on day 5 p.i. and between days 5 and 8 p.i., respectively. Between days 5 and 8 p.i. most animals had pyogranulomatous myocarditis involving atria and ventricles and some also showed pyogranulomatous valvular endocarditis. In these organs there was not expression of selectins, and the basal expression of ICAM-1 on the adrenal capillary network and on the endocardium and myocardial capillaries and venules was clearly up-regulated after infection. Venules and capillaries adjacent to inflammatory lesions in the heart had intense ICAM-1 expression.

Discussion

This report describes the up-regulation of ICAM-1 and the *de novo* expression of endothelial E- and P-selectin during the course of experimental murine listeriosis. After an early, generalized expression of endothelial adhesion molecules in the liver, spleen, and CNS, a further regulation occurred specifically within areas of inflammation. The initial generalized up-regulation of ICAM-1 expression and P-selectin induction in the studied organs were already demonstrable on day 1 p.i. At this time, inflammatory lesions were not evident, but *L. monocytogenes* was systemically disseminated as microbiological counts of blood, liver, and spleen revealed. This suggests a systemic nonspecific endothelial activation that may allow leukocytes to scavenge the organism to find the inflammatory stimulus. During systemic *L. monocytogenes* infection, the liver and spleen are the major target organs for initial bacterial replication and inflammatory cell recruitment, especially neutrophils.[1,8] In the liver, a marked up-regulation of ICAM-1 occurred on day 1 p.i. along all sinusoidal endothelial cells at the same time that P-selectin was induced in postsinusoidal and portal veins but not in the sinusoid. The absence selectin expression on sinusoidal endothelial cells suggests that these molecules are not necessary to mediate the "rolling" phenomenon of leukocytes on the sinusoids of the liver. This is in agreement with other study where P-selectin was shown not to be involved in the initial neutrophil sequestration in hepatic sinusoids during endotoxemia.[16] However, in the same study, P-selectin was critically involved in neutrophil margination and diapedesis in centrilobular and portal veins of mice inoculated with interleukin-1, endotoxin, or tumor necrosis factor-α. Thus, the high up-regulation observed during our experiment suggests that sinusoidal ICAM-1 expression may play a crucial role in the early migration of neutrophils and macrophages to the liver. Furthermore, ICAM-1 has been shown to be essential for the neutrophil-dependent injury of hepatocytes seen during endotoxemia in galactosamine-sensitized mice.[17] After the extensive ICAM-1 sinusoidal expression that lasted to day 3 p.i., only sinusoidal up-regulation surrounding inflammatory lesions and portal vessels was detected through the experiment. This more restricted expression suggests that the organism may direct and recruit inflammatory cells to those places where *Listeria* has been able to survive and multiply. This expression was maximum on day 5 p.i., when bacterial replication and inflammation in the liver were most pronounced. Intense periportal inflammation and the presence of *Listeria* free and within inflammatory cells in the lumen of bile ducts could be seen at the same time that expression of ICAM-1 was induced in biliary epithelial

cells, maybe reflecting a mechanism to reduce both inflammation and bacterial burden from the liver by bile excretion. It has been shown that during the period of maximal hepatic bacterial replication in systemic murine listeriosis, *Listeria* is eliminated through the biliary system.[18]

As in the liver, no expression of any selectin was detected through the experiment in splenic red pulp sinusoidal cells, and only up-regulation of ICAM-1 was demonstrable. The sinusoidal nature of the vascular system in the spleen where blood cells are allowed to have intimate contact with endothelial cells and macrophages in the marginal sinuses of the red pulp could explain, in the same manner that in the liver, the absence of selectin expression and the no necessity of the selectin-mediated rolling in this organ. In lymph nodes even though the immunohistochemical reaction was faint, expression of both P-selectin and ICAM-1 was correlated with leukocyte recruitment to lymph nodes through high endothelial venules.

CNS involvement is a common feature during listeriosis of humans and other animal species.[6,19] However, neither the precise mechanism of leukocyte migration across the blood-brain-barrier nor the signals attracting leukocytes to the CNS are completely understood. In this study, and a previous report,[6] the occurrence of CNS lesions was a late phenomenon after infection and was coincident with the onset of a period of bacteremia. As previously reported, persistent bacteremia is required for the invasion of the murine CNS by *L. monocytogenes* during experimental murine listeriosis using the intravenous route of infection.[2] In our experiment, and in the same way as in the liver and spleen, induction and up-regulation of P-selectin and ICAM-1 in the CNS occurred on day 1 p.i. However, neither inflammation nor *Listeria* antigen were evident until day 3 p.i., when animals showed slight pyogranulomatous leptomeningitis associated with *Listeria* antigen. Interestingly, leptomeningeal inflammation was more evident in the hippocampal sulcus where endothelial P-selectin and ICAM-1 as well as meningeal ICAM-1 were strikingly up-regulated at the time lesions appeared. This indicates that both molecules may play a key role in the recruitment of leukocytes and development of meningitis during experimental murine listeriosis. Supporting these findings, after intracerebral lipopolysaccharide (LPS) inoculation, neutrophil cuffing around ICAM-1-positive vessels in the hippocampal sulcus,[20] and P-selectin expression in almost all veins and venules in the leptomeninges were observed.[21] Contribution of endothelial selectins to the development of meningitis has been shown in a cytokine-induced meningitis model in mice deficient for P- and E-selectin.[22] ICAM-1 has also been reported to be critically involved in the early phase of bacterial meningitis in rats after intracisternal inoculation of pneumococcal cell wall.[27] Expression of ICAM-1 in the subarachnoid connective tissue has been previously shown after LPS inoculation of mice and could contribute to leukocyte adhesion and migration along the subarachnoid space after vascular extravasation.[24] In our experiment ventriculitis/choroiditis followed leptomeningitis and was not associated with P-selectin and ICAM-1 expression on the choroid sinusoidal endothelial cells but with the expression of ICAM-1 on choroidal and ependymal cells. Furthermore, *Listeria* was first detected in association with meningeal inflammation whereas choroid plexus did not show any lesion at that time. This suggests that in this model, inflammatory cells first enter the subarachnoid space through meningeal vessels and not through the choroid plexus as it had been previously suggested.[6] Similar results regarding the time-course of CNS inflammation has been previously reported after intracerebral infection with *L. monocytogenes*.[25] Although our results suggest the possible route taken by leukocytes to reach the subarachnoid space, the way through which *Listeria* arrives to the CNS remains unclear. It has been suggested that some neurotropic bacteria gain access to the cerebrospinal fluid (CSF) through the choroid plexus carried by bacteria-laden macrophages.[6,23] In our conditions, it seems unlikely that *L. monocytogenes* enters the CNS through the choroid plexus, suggesting that probably reaches the CSF through meningeal vessels. In fact, early lesions affecting the ventricular system respected the integrity of the choroidal epithelial cells without apparent inflammatory exocytosis through them, and consisted of an accumulation of inflammatory cells together with numerous *Listeria* in the ventricles. The time course and the histopathological characteristics of the observed lesions indicate that the development of choroiditis could be the consequence of passive accumulation of *Listeria* free and within phagocytes from the subarachnoid space. Furthermore, inflammatory cells could reach the ventricular space from periventricular vessels, which had marked ICAM-1 expression associated with margination and diapedesis when ventricular inflammation was present. Constitutive expression of ICAM-1 on choroid plexus epithelial cells has been reported to function as a costimulatory molecule in antigen presentation and to maintain the proper scavenger function of epiplexus cells.[24,26] Intense inflammation in the CSF could be responsible for the marked up-regulation of ICAM-1 epithelial expression on the choroid plexus as well as the induction on some ependymal cells promoting the adhesion of leukocytes helping them to move along the ventricles to scavenge their surface.

As the CNS, the heart was a commonly affected organ during the late phase of the infection. Endothelial expression of ICAM-1 seems to be involved in the development of myocardial inflammatory infiltrates, which could play an important role on mortality during systemic murine listeriosis.

In summary, strong up-regulation of P-selectin and ICAM-1 occurs during the course of experimental murine listeriosis. This expression is correlated with leukocyte recruitment during murine listeriosis, especially to the liver and CNS. Expression of P-selectin and up-regulation of ICAM-1 in meningeal vessels, especially in those located in the hippocampal sulcus, associated with the initial meningeal inflammation suggest that leukocytes probably reach the CNS through these vessels during experimental murine listeriosis. Additional studies are needed to find out the route taken by *L. monocytogenes* to enter the CNS.

References

1. McKaness GB: Cellular resistance to infection. J Exp Med 1962, 116:381–406
2. Berche P: Bacteremia is required for invasion of the murine central nervous system by Listeria monocytogenes. Microbiol Pathog 1995, 18:323–326
3. Conlan JW: Early pathogenesis of Listeria monocytogenes infection in the mouse spleen. J Med Microbiol 1996, 44:295–302
4. Marco AJ, Altimira J, Prats N, López S, Dominguez L, Domingo M, Briones V: Penetration of Listeria monocytogenes in mice infected by the oral route. Microbiol Pathog 1997, 23:255–263
5. Marco AJ, Prats N, Ramos JA, Briones V, Blanco M, Dominguez L, Domingo M: A microbiological, histopathological, and immunohistological study of the intragastric inoculation of Listeria monocytogenes in mice. J Comp Pathol 1992, 107:1–9
6. Prats N, Briones V, Blanco M, Altimira J, Ramos JA, Dominguez L, Marco A: Choroiditis and meningitis in experimental murine infection with Listeria monocytogenes. Eur J Clin Microbiol Infect Dis 1992, 11:744–777
7. Conlan JW: Critical roles of neutrophils in host defense against experimental systemic infections of mice by Listeria monocytogenes, salmonella typhimurium, and Yersinia enterocolitica. Infect Immun 1997, 65:630–635
8. Czuprynski CJ, Brown JF: Treatment with the antigranulocyte monoclonal antibody RB6–8C5 impairs resistance of mice to gastrointestinal infection with Listeria monocytogenes. Infect Immun 1996, 64: 3946–3949
9. Cronstein BN, Weissmann G: The adhesion molecules of inflammation. Arthritis Rheum 1993, 36:147–157
10. Lasky L: Selectins: interpreters of cell-specific carbohydrate information during inflammation. Science 1992, 258:964–969
11. Springer TA: Adhesion receptors of the immune system. Nature 1990, 346:425–434
12. López S, Borràs D, Juan-Sallés C, Prats N, Domingo M, Marco A: Immunohistochemical detection of adhesion molecules intercellular adhesion molecule-1 and E-selectin in formalin-fixed, paraffin-embedded mouse tissues. Lab Invest 1997, 77:543–544
13. Domingo M, Ramos JA, Dominguez L, Marco AJ: Demonstration of Listeria monocytogenes with the PAP technique in formalin fixed and paraffin embedded tissues of experimentally infected mice. J Vet Med 1986, 33:537–542
14. Henseleit U, Steinbrink K, Goebeler M, Roth J, Vestweber D, Sorg C, Sunderkötter C: E-selectin expression in experimental models of inflammation in mice. J Pathol 1996, 180:317–325
15. McEver RP, Beckstead JH, Moore KL, Marshall-Carlson L, Bainton DF: GMP-140, a platelet α-granule membrane protein, is also synthesized by vascular endothelial cells and is localized in Weibel-Palade bodies. J Clin Invest 1989, 84:92–99
16. Essani NA, Fisher MA, Simmons CA, Hoover JL, Farhood A, Jaeschke H: Increased P-selectin gene expression in the liver vasculature, and its role in the pathophysiology of neutrophil-induced liver injury in murine endotoxin shock. J Leukocyte Biol 1998, 63:288–296
17. Essani NA, Fisher MA, Farhood A, Manning AM, Smith CW, Jaeschke H: Cytokine-induced upregulation of hepatic intercellular adhesion molecule-1 messenger RNA expression and its role in the pathophysiology of murine endotoxin shock and acute liver failure. Hepatology 1995, 21:1632–1639
18. Briones V, Blanco M, Marco AJ, Prats N, Fernández-Garayzabal JF, Suarez G, Domingo M, Domínguez L: Biliary excretion as possible origin of Listeria monocytogenes in fecal carriers. Am J Vet Res 1992, 53:191–193
19. Schuchat A, Swaminathan B, Broome CV: Epidemiology of human listeriosis. Clin Microbiol Rev 1991, 4:169–183
20. Bell MD, Perry VH: Adhesion molecule expression on murine cerebral endothelium following the injection of a proinflammagen or during acute neuronal degeneration. J Neurocytol 1995, 24:695–710
21. Gotsch U, Jäger U, Dominis M, Vestweber D: Expression of P-selectin on endothelial cells is upregulated by LPS and TNF-α in vivo. Cell Adhes Commun 1994, 2:7–14
22. Tang T, Frenette PS, Hynes RO, Wagner DD: Cytokine-induced meningitis is dramatically attenuated in mice deficient in endothelial selectins. J Clin Invest 1996, 97:2485–2490
23. Williams AE, Blakemore WF: Pathogenesis of meningitis caused by Streptococcus suis type 2. J Infect Dis 1990, 162:474–481
24. Endo H, Sasaki K, Tonosaki A, Kayama T: Three-dimensional and ultrastructural ICAM-1 distribution in the choroid plexus, arachnoid membrane and dural sinus of inflammatory rats induced by LPS injection in the lateral ventricles. Brain Res 1998, 793:297–301
25. Seebach J, Bartholdi D, Frei K, Spanaus K, Ferrero E, Widmer U, Isenmann S, Strieter RM, Schwab M, Pfister H, Fontana A: Experimental Listeria meningoencephalitis. J Immunol 1995, 155:4367–4375
26. Steffen BJ, Butcher EC, Schulz M, Engelhardt B: Icam-1, Vcam-1, and MadCAM-1 are expressed on choroid plexus epithelium but not endothelium and mediate binding of lymphocytes in vitro. Am J Physiol 1996, 148:1819–1838
27. Weber JR, Angstwurm K, Bürger W, Einhaüpl KM, Dirnagl U: Anti Icam-1 (CD54) monoclonal antibody reduces inflammatory changes in experimental bacterial meningitis. J Neuroimmunol 1995, 63:63–68