Veterinary Microbiology 166 (2013) 438–449

Contents lists available at SciVerse ScienceDirect

Veterinary Microbiology

journal homepage: www.elsevier.com/locate/vetmic

Altered expression of adhesion molecules on peripheral blood leukocytes in feline infectious peritonitis

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ARTICLE INFO

Article history:
Received 13 February 2013
Received in revised form 19 June 2013
Accepted 24 June 2013

Keywords:
FIP
Adhesion molecules
Peripheral blood leukocytes
Flow cytometry

ABSTRACT

Feline infectious peritonitis (FIP) is a fatal, coronavirus-induced systemic disease in domestic and wild felids. The pathology associated with FIP (multifocal granulomatous vasculitis) is considered to be elicited by exaggerated activation and subsequent extravasation of leukocytes. As changes in the expression of adhesion molecules on circulating leukocytes precede their margination and emigration, we reasoned that the expression of leukocyte adhesion molecules may be altered in FIP. In present study, the expression of principal adhesion molecules involved in leukocyte transmigration (CD15s, CD11a, CD11b, CD18, CD49d, and CD54) on peripheral blood leukocytes from cats with naturally occurring FIP ($n = 15$) and controls ($n = 12$) was quantified by flow cytometry using a formaldehyde-based rapid leukocyte preparation technique. T- and B-lymphocytes from FIP patients exhibit higher expression of both subunits (CD11a and CD18) composing the β_2 integrin lymphocyte function-associated antigen (LFA)-1. In addition, the expression of the α_4 subunit (CD49d) of the β_1 integrin very late antigen (VLA)-4 was elevated on B-lymphocytes from FIP patients. The expression of CD11b and CD18, that combine to form the β_2 integrin macrophage-1 antigen (Mac-1), was elevated on monocytes, whereas the density of CD49d was reduced on this population in FIP. Granulocytes of FIP cats displayed an increased expression of the α chain of Mac-1 (CD11b). These observations suggest that leukocytes from FIP patients show signs of systemic activation causing them to extravasate into surrounding tissues and ultimately contribute to pyogranuloma formation seen in FIP.

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1. Introduction

Feline infectious peritonitis, first described in 1963, is a highly fatal, progressive, and immunopathological disease of domestic and wild *Felidae* (Holzworth, 1963). FIP occurs worldwide and is currently one of the leading infectious causes of death in cats. The causative agent of FIP, feline coronavirus (FCoV), is ubiquitous in virtually all cat populations, with seropositivity of up to 90% depending on environment and geographical area. From the seropositive cats, 5–12% eventually develop FIP (Addie and Jarrett, 1992). FCoV is an enveloped, single-stranded, positive-sense RNA virus belonging to the *Coronaviridae* family within the order of the *Nidovirales* (Gorbalenya et al., 2006). FCoVs are classified into two serotypes (I and II) according to the amino acid sequence of the spike protein (Herrewegh et al., 1998). In addition, each serotype can be further divided into two distinctly different pathotypes, based on their pathogenicity in cats. The most common pathotype in the field, feline enteric coronavirus (FECV), causes a mild, often unapparent enteric infection. In sharp contrast, infection with the virulent pathotype, designated

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0378-1135/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.vetmic.2013.06.027

feline infectious peritonitis virus (FIPV), manifests as a devastating, highly lethal systemic disease called FIP (Pedersen, 1987). FIP is characterized by fibrinous to granulomatous serositis, often with protein-rich effusions in body cavities, and (pyo)granulomatous inflammatory lesions in various organs (Weiss and Scott, 1981a,b). In the heterogeneous and sometimes confusing histopathological picture of FIP one distinct feature stands out: the multifocal granulomatous vasculitis, restricted to small and medium-sized veins. Morphologically, the vasculitis is typified by a venous and perivenous circular cell infiltrate dominated by monocytes/macrophages intermingled with a few neutrophils and lymphocytes (Kipar et al., 1998). The vasculitis has previously been regarded to be induced by a type III hypersensitivity reaction, involving the deposition of immune complexes within venules leading to complement activation (Jacobsegeels et al., 1982; Pedersen and Boyle, 1980). However, Kipar and co-workers demonstrated that the morphology, cellular composition, and distribution of the vascular lesions in FIP differs significantly from immune-complex-mediated vascular inflammatory processes. Furthermore, the demonstration of FCoV antigen within intravascular leukocytes and among cells in the FIP granulomas indicates that the phlebitis is initiated by activated and FCoV-infected circulating monocytes. Excessive numbers of activated monocytes will emigrate out of the blood circulation and accumulate perivenously. The exaggerated extravasation is associated with enhanced local release of matrix metalloproteinases B (MMP 9) leading to endothelial barrier dysfunction (Kipar et al., 2005). Considering the importance of leukocyte extravasation in the pathogenesis of FIP, comparatively few studies have been aimed at investigating this key pathogenic event. A crucial step in the process of leukocyte recruitment into the parenchyma is the adherence of circulating leukocytes to vascular endothelial cells (EC), which is facilitated by adhesion molecules expressed on the surface of participating cells. Based on their biochemical properties and molecular structure, these adhesion molecules have been grouped into three gene families: the selectins, the integrins, and the immunoglobulin (Ig) supergene family (Carlos and Harlan, 1994). The initial adhesive interaction involves the participation of endothelial selectins that bind with sialyl lewis X (sLex, CD15s) carbohydrate moieties, which can be found on the terminal domains of glycoproteins expressed on the leukocyte surface. Because of its relative low affinity nature, this binding results under hydrodynamic shear flow in leukocyte rolling along the vessel wall. The transiently bound leukocytes are subsequently activated on encountering immobilized chemokines at the endothelial surface. This activation step enables the strengthening of the adhesive forces and leukocytes become firmly attached to the endothelium. This firm adhesion is achieved by the interplay between integrins and Ig supergene family receptors (Ebnet and Vestweber, 1999). The integrins are heterodimer glycoproteins consisting of two non-covalently associated dissimilar proteins designated α and β chain. The integrins are divided into different groups dependent on the common β chain. The β_2 (CD18) subunit combines with α_L (CD11a) subunit to build lymphocyte function-associated antigen (LFA)-1 or with α_M (CD11b) to form macrophage-1 antigen (Mac-1). They are responsible for interactions with intercellular adhesion molecule (ICAM)-1 (CD54), present constitutively on EC and markedly induced during inflammation. The β_1 integrins named very late antigen (VLA) share a common β_1 (CD29) chain that can be linked with a number of α chains. The VLA-4 receptor contains α_4 subunit (CD49d) and is one of the β_1 integrins responsible for leukocyte extravasation due to its interaction with vascular cell adhesion molecule (VCAM)-1 present on activated endothelium. Both ICAM-1 and VCAM-1 belong to the Ig supergene family and are built of several immunoglobulin domains (Barreiro and Sanchez-Madrid, 2009; Carlos and Harlan, 1994).

Given the large number of leukocytes in FIP lesions, we hypothesized that FIPV infection could alter the expression of adhesion molecules on leukocytes. This might favor leukocyte–endothelial adherence and subsequent migration, thereby inducing endothelial cell damage and contributing to the pathogenesis of FIP. Despite their critical role in the pathological outcome, the expression of adhesion molecules on peripheral leukocytes during FIPV infection remains mainly undescribed. Identification of adhesion molecules with altered expression in FIP will not only provide invaluable insights to further elucidate the pathogenesis but this essential data can also assist in the development of more accurate diagnostic methods.

Therefore, the principal aim of present study was to quantify the expression of adhesion molecules on the main peripheral leukocyte populations in FIP patients and healthy controls by flow cytometry using the formaldehyde-based rapid leukocyte preparation technique (Hamblin et al., 1992). With a panel of thoroughly validated monoclonal antibodies, the main adhesion molecules associated with leukocyte transendothelial migration were studied: CD15s, CD11a, CD11b, CD18, CD49d, and CD54.

2. Materials and methods

2.1. Patients and controls

Fifteen cats, naturally infected with FCoV and clinically strongly suspected of FIP (based on cat profile, clinical signs and blood and/or exudate examination) were included in this study. The characteristics and pathological findings of these cats are listed in Table 1. In all cases, the presumptive diagnosis of FIP was confirmed by post-mortem examinations that comprised necroscopy, histology and immunohistochemistry for FCoV-antigens. Patients were not receiving corticosteroids or other immunosuppressive therapy. Serology for feline immunodeficiency virus (FIV) and for feline leukaemia virus (FeLV) was performed using a commercially available ELISA kit (Witness® FeLV-FIV, Synbiotics Corporation, San Diego, CA, USA) according to the procedure advised by the manufacturer. All cats were confirmed not to be infected with FIV and FeLV.

A control group of twelve specific pathogen free (SPF) cats were permanently kept at the animal facility of the Faculty of Veterinary Medicine of Ghent University. At the time of sampling, these cats were clinically healthy and did

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