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Different susceptibilities of PECAM-deficient mouse strains to spontaneous idiopathic pneumonitis

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Abstract

Platelet Endothelial Cell Adhesion Molecule (PECAM) is an adhesion and signaling molecule used for leukocyte extravasation. We have generated two strains of PECAM-deficient mouse, one in the original C57BL/6 and a second by backcrossing nice generations into the FVB/n strain. The FVB/n strain has reduced responses in models of acute inflammation. We show here that this strain is also susceptible to a chronic pneumonia which leads to pulmonary fibrosis. In contrast, PECAM-deficient C57BL/6 mice do not develop this lung disease and have normal responses in acute models of inflammation. This demonstrates that PECAM-dependent and -independent mechanisms are found in both acute and chronic inflammation. Further, the PECAM-deficient FVB/n strain has many pathologic similarities to the human disease Idiopathic Pulmonary Fibrosis, suggesting that similar molecular mechanisms may play a role in human disease.

Introduction

Leukocyte extravasation in response to inflammatory signals on the endothelial cells that line the blood vessels is one of the earliest steps in the response to a pathogen. Platelet Endothelial Cell Adhesion Molecule (PECAM) is an adhesion molecule used by leukocytes, especially monocytes and neutrophils, to exit blood vessels in this process. PECAM is a 130-kDa member of the Immunoglobulin superfamily that is expressed both on leukocytes and in the junctions between the endothelial cells that line all blood vessels. Leukocyte migration across the blood vessel wall involves interaction of PECAM on the leukocyte with the PECAM at the endothelial cell junction (Muller, 1995; Muller, 1999; Newman, 1997).

On the other hand, the immune response must be terminated after the infection is cleared to prevent further tissue damage, and PECAM has also been shown to regulate programmed cell death (apoptosis) (Bird et al., 1999; Evans et al., 2001; Gao et al., 2003; Noble et al., 1999). PECAM has two cytoplasmic Immunoreceptor Tyrosine Inhibitory Motif (ITIM) domains for intracellular signaling (Newman et al., 2001; Newton-Nash and Newman, 1999). When PECAM on live cells engages macrophage PECAM, phagocytosis is prevented. Signaling through the cytoplasmic tail and the ITIM domains is important for both preventing ingestion of live leukocytes and increasing resistance to apoptosis (Brown et al., 2002; Gao et al., 2003). Thus, PECAM function is important for both promoting and terminating inflammation.

PECAM is important for leukocyte responses to inflammation, but there are also PECAM-independent pathways. Blocking PECAM prevents extravasation of a majority of monocytes and neutrophils in vitro and in vivo. However, a minor population (~20–25%) of both monocytes and neutrophils can cross by PECAM-independent mechanisms (Muller, 1999). Further, when mice genetically deficient for PECAM were first derived in the C57BL/6 strain, they were able to use PECAM-independent mechanisms to mobilize normal numbers of monocytes and neutrophils in several models of inflammation (Duncan et al., 1999). While these PECAM-deficient mice were subsequently found to have some deficiencies in their inflammatory responses in certain models (Carrithers et al.,

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2005; Graesser et al., 2002; Maas et al., 2005; Solowiej et al., 2003; Thompson et al., 2001), C57BL/6 mice appear to be unique in their relative insensitivity to PECAM blockade. All other mouse strains tested have significantly reduced inflammatory reactions when PECAM is genetically deficient or blocked in wild-type mice using antibodies. The other genetically PECAM−/− mouse strain, FVB/n, fails to mobilize monocytes and neutrophils in models of peritonitis and dermatitis (Schenkel et al., 2004). Studies in the literature show that inflammation can be blocked by anti-PECAM antibodies in strains like CD2F1 (Bogen et al., 1994), AKR/J (Bogen et al., 1994), AND, a T lymphocyte transgenic derived from SJL (Qing et al., 2001), and DBA1/J (Ishikaw et al., 2002). We have confirmed the result in FVB/n, SJL, and Swiss Webster (outbred) mice (Schenkel et al., 2004). In rats treated with anti-PECAM antibodies, neutrophil emigration was also blocked (Vaporciyan et al., 1993), and leukocyte adhesion to synovial blood vessels was blocked in a model of rheumatoid arthritis (Decking et al., 2001). The amount that inflammation was reduced in these in vivo studies is similar to what we have observed with human leukocytes in vitro (Muller, 1995; Muller, 1999). Thus, PECAM-dependent mechanisms are used widely in mice, rats, and humans for leukocyte trafficking.

PECAM is highly expressed on lung vasculature (Marszalek et al., 2000; Muller et al., 1989). A high proportion of PECAM−/− FVB/n mice in clean veterinary facilities spontaneously develop a chronic lung disease described in this report, while their wild-type littermates are unaffected. Further, the C57BL/6 PECAM−/− mice do not develop chronic lung disease. Thus, PECAM-independent mechanisms must play a significant role in the resistance of C57BL/6 mice to a chronic and ultimately fatal pneumonia.

Materials and methods

Mouse strains

C57BL/6 or FVB/n wild-type and PECAM knockout mice were raised and housed at Weill Medical College of Cornell University. PECAM-deficient mice in the C57BL/6 background have been previously described (Duncan et al., 1999; Schenkel et al., 2004). PECAM-deficient mice in the FVB/n background were generated by nine successive backcrosses (Schenkel et al., 2004). Mice were kept in specific pathogen-free housing. Sentinel mice were screened for antibodies to most common murine viral, bacterial, fungal, and parasitic infections. Over 3 years, only one sentinel mouse was positive for Mouse Hepatitis Virus, PECAM-deficient mice were tested but did not have detectable antibodies.

Pathologic examination

At necropsy, animals were dissected to expose the lungs. Gross observations were noted, and the lungs were removed. Lungs were fixed in 10% buffered formalin and submitted for embedding in paraffin. Sections were mounted and stained with hematoxylin and eosin stains. Blinded scoring of the specimens was done on a Zeiss microscope, and the code broken after all scoring was completed. The clinical scoring system was as follows: No pathology = 0, Multifocal inflammation = +, Inflammation/Fibrosis in 25% of lung = ++, Inflammation/Fibrosis in 25–50% of lung = ++, Inflammation/Fibrosis in > 50% of lung = +++.

Detection of pathogens

Bronchoalveolar lavage fluid was collected as follows. The trachea was exposed using sterile technique and lavaged with sterile saline using an 18-gauge feeding needle attached to a 1-cm^3 syringe. This fluid was cultured for aerobic bacteria. Pieces of lung tissues were also cultured for aerobic bacteria. Other pieces were stained using silver stains to identify any fungal or bacterial infections. Culture and staining were performed by veterinary services at Weill Medical College of Cornell University.

Western blot for Ym1

Lungs were prepared lavaged as described. Lavaged cells were counted and pelleted for centrifugation. Supernatants and cell pellets were lysed in lysis buffer for SDS polyacrylamide gel electrophoresis containing 2% SDS, 12% sucrose, 0.01% Bromphenol Blue in 50 mM carbonate buffer pH 8.6 ± 5% β-mercaptoethanol. Additionally, lungs were minced and lysed in a lysis buffer containing 10 mM HEPES pH 7.9, 1.5 mM MgCL2, 10 mM KCl, 0.5 mM DTT, 0.1% NP-40, and Eukaryotic Protease Inhibitor Cocktail (4-(2-aminoethyl) benzenesulfonyl fluoride, pepstatinA, E-64, bestatin, leupeptin, and aprotinin Sigma, St. Louis, MO) The lysate was centrifuged at high speed, and the insoluble pellets and soluble supernatant were diluted in lysis buffer (Neville, 1971). Proteins were separated by gel electrophoresis on 4–20% polyacrylamide gradient gels in Tris–SDS buffer. The lysate was transferred to PVDF membranes by semi-dry electrophoresis at 25 W for 1 h. The membranes were blocked with 5% bovine serum albumin for 1 h in Tris-buffered saline +0.1% Tween-20 (TBST). Rabbit anti-serum against murine Ym1 (Harbord et al., 2002) was incubated with the membranes in TBST, washed to remove unbound antibody, incubated with goat anti-rabbit secondary antiserum conjugated to horseradish peroxidase for 1 h, and washed to remove unbound antibodies. The membranes were then incubated with CDP-Star chemiluminescent substrate (Perkin Elmer, Norwalk, CT) and placed on photographic films for detection.

Immunohistochemistry

Four-micrometer paraffin sections were deparaffinzed then treated with hydrogen peroxide and then blocked with Serum Free Protein Block (DakoCytomation, Carpinteria, CA). Apoptotic cells were immunohistochemically stained with a polyclonal rabbit anti-Cleaved Caspase-3 (Asp175) antibody (Cell Signaling Technology, Beverly, MA). A pretreatment of heat-induced epitope retrieval in Target Retrieval Solution, pH 9.0 (DakoCytomation, Carpinteria, CA) was utilized. The antibody was incubated for 30 min at room temperature and then visualized using Envision + rabbit (DakoCytomation, Carpinteria, CA) followed by DAKO Liquid DAB+ (DakoCytomation, Carpinteria, CA). Cells positive for F4/80 were immunohistochemically stained with a monoclonal rat anti-mouse F4/80 (Serotec, Raleigh, NC). Prior to primary incubation, the sections were treated with proteinase K (DakoCytomation, Carpinteria, CA). The antibody was incubated for 30 min at room temperature. After incubation with primary antibody, the tissue sections were sequentially incubated with biotinylated rabbit anti-rat immunoglobulin (DakoCytomation, Carpinteria, CA) and then additionally treated with Envision + Rabbit (DakoCytomation, Carpinteria, CA). Staining was developed with Liquid DAB+ (DakoCytomation, Carpinteria, CA). YM-1 was localized with a rabbit anti-murine Ym1 (Harbord et al., 2002), and Wide Spectrum Screen Keratin (DakoCytomation, Carpinteria, CA) was used to localize cytokeratin, the antibodies were incubated for 30 min at room temperature and then additionally treated with Envision + Rabbit (DakoCytomation, Carpinteria, CA). Staining was developed with Liquid DAB+ (DakoCytomation, Carpinteria, CA).

Statistics

Survival curves between C57BL/6 and FVB/n PECAM−/− mice was done with Kaplan–Meier Survival Analysis (JMPin Software, SAS Institute, Cary, NC).

Results

The first evidence for a role of PECAM in lung inflammation came from the survival of PECAM−/− FVB/n strain mice in our breeding colony. After derivation of this strain, we initially