Surface Marker Analysis of the Vascular and Epithelia Lesions in Cattle with Sheep-Associated Malignant Catarrhal Fever

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ABSTRACT. Surface marker analysis of the vascular and epithelial lesions in cattle with sheep-associated malignant catarrhal fever (MCF) were done by immunohistochemistry using 7 monoclonal antibodies. MHC class I and II antigens were expressed in the degenerated portion of the vascular walls in addition to infiltrated leukocytes. The major population of mononuclear cells in these lesions were phenotypically macrophages. The other cells had BoCD4 or BoCD8, but rarely γδ T cell markers. These results suggest involvement of MHC restriction and macrophages, in addition to autoaggressive cytotoxic T lymphocytes, in the development of MCF vascular lesion.—KEY WORDS: BoCD4, BoCD8, macrophage, malignant catarrhal fever, MHC.

Malignant catarrhal fever (MCF) is a sporadic but fatal disease of cattle and other ruminants [12]. Wildebeest-derived MCF in Africa is caused by alcelaphine herpesvirus 1. Another form, sheep-associated (SA) MCF occurs in relation to sheep lambing in the other countries, but the causal agent has not been identified. The lesions are similar in both forms of MCF, and are composed of systemic vasculitis and epithelial necrosis infiltrated with mononuclear leukocytes as well as interstitial infiltration of mononuclear leukocytes in various organs. The mechanism of MCF lesions still remains in a mystery, but the virtual absence of viral cytopathology, antigens and virions in any of the MCF lesions suggest primarily cell-mediated immune responses [12]. In vitro investigations suggested that lymphoblastoid cell lines with natural killer (NK) cell or lymphokine activated killer (LAK) characteristics are involved in the pathogenesis of MCF [3, 13, 14]. In vivo, the role of cytotoxic T lymphocytes in MCF has been inferred by phenotyping of T lymphocytes in MCF lesions [5, 11]. The present report documents expression of major histocompatibility complex (MHC) antigens in the vascular or epithelial lesions of MCF, and the predominance of macrophages in these lesions, as well as BoCD4+ or BoCD8+ T cells.

MATERIALS AND METHODS

Animal: Two cattle (3 months old: No. 4195, 1.5-year old: No. 4197) with pyrexia, corneal opacity and mucopurulent discharge around the mouth and rhinal orifices, were killed and necropsied for routine diagnosis. In both cases SA-MCF was suspected from clinical signs and episodes of contact with suckling lambs within the previous 5 months.

Surface marker analysis of MCF lesions: Specimens of

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the kidney, urinary bladder, muzzle, rumen, liver, heart, brain, rete mirabile around the hypophysis and lymph nodes were frozen in −70°C in normal hexane at necropsy. Visceral organs and cervical and mesenteric lymph nodes from apparently normal calves were also frozen as controls. Cryostat sections were fixed in cold acetone, and stained with the following monoclonal antibodies (mAbs) (Table 1): DH59B (monocytes/macrophages), IL-A11 (BoCD4), IL-A51 (BoCD8), IL-A29 (BoWC1; γδ T), CACTB14A (BoWC2 (N6); γδ T), IL-A88 (MHC class I), J-11 (MHC class II) [4, 6, 8, 15], and a kit for avidin-biotin complex (ABC) immunoperoxidase methods (Vectastain Elite, Vector, U.S.A.). CACTB14A and DH59B were purchased from VMRD (U.S.A.) and the other mAbs were provided by ILRAD (Kenya). Reaction of the mAbs in MCF lesions was scored under microscopy (Table 1).

RESULTS

Diagnosis of MCF was confirmed by the presence of the pathognomonic lesions of systemic vasculitis and epithelial necrosis. Vascular lesions consisted of focal degeneration in the mural walls, intense infiltration of mononuclear cells in the intima, media and adventitia, and swelling of endothelial cells (Fig. 1). Epithelial cells of the urinary bladder and upper digestive tract were necrotic or degenerated and infiltrated with mononuclear cells. Systemic perivascular or interstitial infiltration of mononuclear cells in the brain and visceral organs were present.

The pattern designated by the mAb panel was similar in the epithelial and vascular lesions of the both cases (Table 1). Degenerated portion of tunica media in the vascular lesion was positive for MHC class I recognized by IL-A88, in addition to the intima and infiltrated mononuclear cells in the vascular and epithelial lesions (Fig. 2). Intima but not tunica media was reactive with IL-A88 in normal artery. Similarly, degenerated portions of the vascular...
Table 1. Reaction of monoclonal antibodies in MCF lesions

<table>
<thead>
<tr>
<th>Monoclonal Specificity</th>
<th>Vascular lesions</th>
<th>Epithelial lesions</th>
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<tbody>
<tr>
<td></td>
<td>No. 4195</td>
<td>No. 4197</td>
</tr>
<tr>
<td>antibody</td>
<td>Kidney</td>
<td>Rete mirabilis</td>
</tr>
<tr>
<td>DH59B</td>
<td>Macrophages</td>
<td>ND³</td>
</tr>
<tr>
<td>IL-A11</td>
<td>BoCD4</td>
<td>++</td>
</tr>
<tr>
<td>IL-A51</td>
<td>BoCD8</td>
<td>+++</td>
</tr>
<tr>
<td>IL-A29</td>
<td>BoWC1</td>
<td>+</td>
</tr>
<tr>
<td>CACTB14A</td>
<td>BoWC2 (N6)</td>
<td>++</td>
</tr>
<tr>
<td>IL-A88</td>
<td>MHC I³</td>
<td>+</td>
</tr>
<tr>
<td>J-II</td>
<td>MHC II³</td>
<td>+</td>
</tr>
</tbody>
</table>

a) Not done.
b) Frequency of the positive cells is scored as follows: --: negative, +: nearly 10%, ++: approximately 10-25%, +++: approximately 25-50%, ++++: more than 50%.
c) Unusual over expression in the degenerated tissue means +, because most of the cells are positive.

The predominant infiltrates were phenotypically macrophages. The significance of NK, CTL or LAK cells has been emphasized in relation to immune-mediated condition of MCF lesions [3, 5, 11, 14]. However, macrophages or monocytes had MCF virus infectivity in experimental infection of rabbits [10] and some of infiltrated mononuclear cells in the vascular lesion in MCF of cattle are both phenotypically and electron microscopically macrophages [5, 9].

Among T lymphocytes, BoCD8+ cells were predominant in the present case. This agreed with previous results of the predominance of BoCD8+ cells in vascular and epithelial lesions of MCF in cattle [5, 11], suggesting involvement of CTL. In vitro, the lymphoblastoid cell lines derived from MCF cattle have large granular lymphocyte/NK or LAK-like ability that kill promiscuously co-cultured feeder cells [3, 13]. The cell lines derived from cattle containing MCF agent have a surface marker of BoCD4 or BoCD8 [2]. γδ T lymphocytes were present in MCF lesions, but the ratio of these cells did not increase in either vascular and epithelial lesions. The function and role of γδ T cells in infection is still unclear [7], their role in MCF of cattle might not be significant. However the cell line derived from deer with MCF had T19 antigen [2], which is expressed in mature γδ T lymphocytes [7], suggesting a different condition of MCF pathogenesis in deer and cattle.

Based on previous investigations and present results, the pathogenesis of the vascular lesion of MCF in cattle may be a concurrent condition of MHC restricted reaction of BoCD4+ cells, BoCD8+ cells and macrophages, and autoaggressive nature of BoCD8+ CTL.

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Fig. 1. Vascular lesion with infiltration of mononuclear cells in the rete mirabile of No. 4195. HE, × 160.
Fig. 2. Expression of MHC class I antigen in the vascular lesions (rete mirabile, No. 4195). Degenerative portion of tunica media, infiltrated cells, intima and adventitia are positive, but relatively normal portion (*) of tunica media is negative. ABC, × 100.
Fig. 3. Expression of MHC class II antigen in the vascular lesions (kidney, No. 4195). Degenerative portion of tunica media is weakly positive and infiltrated cells in the adventitia are positive. ABC, × 160.
Fig. 4. Many of the infiltrated cells in the vascular lesion are macrophages as stained with mAb DH59B (rete mirabile, No. 4195). ABC, × 160.
REFERENCES


