Analysis of Serum Cytokine Profile in a Holstein Heifer with Leukocyte Adhesion Deficiency Which Survived for Long Period

Hajime NAGAHATA1, Katsuro HAGIWARA2, Hidetoshi HIGUCHI1, Rikio KIRISAWA2 and Hiroshi IWAI2

1Departments of Animal Health and 2Veterinary Microbiology, School of Veterinary Medicine, Rakuno Gakuen University, Ebetsu, Hokkaido 069-8501, Japan

(Received 29 June 2001/Accepted 2 April 2002)

ABSTRACT. Serum cytokine levels and their expression of mRNA on neutrophils from a bone marrow (BM) transplanted heifer with leukocyte adhesion deficiency were evaluated. The clinical condition of the affected heifer was relatively stable after BM-transplantation. Persistent hyper γ-globulinemia and increased serum immunoglobulin G (IgG) concentrations were monitored longitudinally. The concentration of interleukin (IL)-1β in serum from the affected heifer ranged from 15.8 to 321.7 ng/ml, and maximum concentration occurred at the time which coincided with peak IL-6. Serum levels of IL-6 ranged from 0.32 to 27.9 ng/ml, and they appeared to be associated with the increment of serum IgG in the affected heifer. mRNAs for IL-1β, IL-6, IL-8 and granulocyte and macrophage colony stimulating factor (GM-CSF) were increased in neutrophils from the affected heifer compared to controls. Persistent hyper γ-globulinemia of the affected heifer appeared to be associated with enhanced mRNA expression for IL-6 and its serum levels. These findings suggest that humoral immunity of the affected heifer is activated and the production of neutrophils appears to be enhanced under the incapability of β2 integrin-mediated functions of phagocytic cells.

KEY WORDS: BLAD, IL-1β, IL-6, IL-8.

Leukocyte adhesion deficiency in Holstein cattle (BLAD) is a hereditary disease characterized by impaired expression of β2 integrin on leukocytes [8,12,17]. Profound abnormalities of leukocytes from cattle with BLAD were elucidated [8,13]. Impaired diapedesis of neutrophils was the characteristic finding seen in inflamed tissues of cattle with BLAD. In most cases of the affected cattle with BLAD, animals died at an early age of 1 month to 12 months due to severe pneumonia and/or diarrhea [13]. In our previous study, we applied bone marrow (BM) transplantation to the BLAD heifer aged at 9 months old, and clinical and immunological studies were performed previously during 26 months after BM transplantation [14]. Thereafter the general clinical conditions of this BM treated heifer were relatively stable for 1.6 years. This BM transplanted heifer appeared to be rare because as long as survived up to 4.6 years old. In addition, the finding of systemic k-amyloidosis in cattle was newly demonstrated in this BLAD heifer by post mortem pathological studies [19]. From these reasons, further information of this heifer appears to be valuable to elucidate the characteristic findings of this affected heifer with leukocyte adhesion deficiency. The aim of this report was to describe the follow up clinical and pathological findings and selected cytokine profiles in the BM-transplanted heifer for further understanding of β2 integrin-deficient host.

MATERIALS AND METHODS

Animals: A 3 year-old Holstein heifer with BLAD that had been previously BM-transplanted at 9 months old was used [14]. The heifer was housed and fed a 16% protein growing ration, hay, minerals and ad libitum water daily. The clinical picture, rectal temperature, heart and respiratory rates were monitored daily. The body condition of the affected heifer was evaluated based on the routine body condition scores [4].

Blood: Ten millilitres of blood was collected from the jugular vein into a tube containing heparin (20 IU/ml). Total and differential leukocyte counts were performed according to standard hematological methods, as described [13].

Protein assay: Total protein was determined by the routine serum protein assay (Biuret method, Wako). Cellulose acetate electrophoresis of serum proteins was carried out according to the standard procedures. Each fraction of serum proteins was quantified densitometrically.

Immunoglobulins: Concentrations of immunoglobulin (Ig)G, IgA, and IgM were measured by use of single radial immunodiffusion assay kits (Saikin Kagaku Inc., Sendai).

Detection of cytokines: The concentrations of IL-1β, IL-6 and TNF-α in the sera from the BLAD were determined by a sandwich enzyme-linked immunoassay (ELISA) using bovine IL-1β, IL-6 and TNF-α specific antibodies. The cytokines used for this assay was a fusion protein of the bovine cytokine sequence with glutathione S-transferase (GST) expressed in Escherichia coli as described by Hagiwara et al. [6]. Affinity-purified polyclonal antibodies from rabbits immunized with recombinant bovine IL-1β, IL-6 or TNF-α were used for the capture element of the ELISA. These antibodies were labelled with biotin by a commercially available kit (Amersham) and used for the detection of the cytokine antibodies, as described previously [6]. The concentrations of cytokines were determined by plotted standard curves using these recombinant cytokines.

The concentration of GM-CSF was determined using a
commercially available assay kit (Quantikine kit, R & D systems).

Detection of cytokine mRNA: To detect cytokine mRNA (IL-1β, IL-6, IL-8 and GM-CSF) in neutrophils from the BLAD heifer, we employed a reverse transcriptase polymerase chain reaction (PCR) method. Total cellular RNAs were extracted from cells using an RNA isolation kit (TRIZOL reagent; GIBCO BRL). The RNAs (1 μg) were reverse-transcribed using ReverTra DashTM (TOYOBO) and random primers (25 pmol/μl) for 10 min at 30°C, 20 min at 42°C, and then the reaction was terminated by incubation at 99°C for 5 min. Cytokine specific cDNAs corresponding to each cytokine sequence were amplified by PCR method [6]. The primers used were the same as described [3, 7, 10, 11]. The PCR products were separated by 1.5% agarose gel electrophoresis and then stained with ethidium bromide.

RESULTS

Clinical course: Changes in the body weight of the heifer from 27 months after BM transplantation showed no increased gain in weight (data not shown). The score of the body condition of the heifer during this period was estimated to be 2.0–2.5, based on the scoring of the routine body condition. Changes in the rectal temperature, and heart and respiratory rates were monitored (Fig. 1). The rectal temperature of the BLAD heifer was generally stable and elevated temperature over 39.5°C was rarely detected from 27 months after BM transplantation until 6 months before death. The heart and respiratory rates were also stable during this period.

Immunoglobulins: Hyper γ-globulinemia gradually developed and persisted (Fig. 2). Levels for serum α- and β-globulins were relatively stable during this period. The concentrations of IgG in serum from the affected heifer were increased gradually from 6 months to 4 years of age (Fig. 3). The mean concentration of IgG in the serum of the affected heifer was 2.4 to 4.5 fold higher when compared to the controls. The concentrations for IgA and IgM in serum from affected heifer were not persistently increased (data not shown).

Cytokines: The concentrations of IL-1β and IL-6 in serum from the BLAD heifer from 6 months to 4 years old were evaluated. The values of serum IL-1β in the BLAD heifer during this interval ranged from 69.4 to 321.7 ng/ml, and the peak value was found in serum from the affected animal at 2 years old. IL-1β levels were undetectable in the controls (Fig. 4). Serum IL-6 in the BLAD heifer ranged from 0.32 to 27.9 ng/ml, and their levels were apparently higher than those of normal controls (Fig. 4). TNF-α in serum from the BLAD heifer, 5 samples from the affected animal, and controls, 5 age-matched normal animals, was undetected.

Cytokine gene expressions: Expression of mRNA for IL-1β, IL-6, IL-8 and GM-CSF was detected on neutrophils from the BLAD-affected heifer aged from 2 to 4 years old, compared to those of controls which showed undetectable levels (Fig. 5).

DISCUSSION

This BM-transplanted heifer with BLAD survived for 4.6 years. Survival to this age is rare in BLAD-affected cattle. The growth rate of the affected heifer was apparently delayed and was associated with an unthrifty body condition. The general clinical condition was relatively stable during the course of life until 1 month before termination. The heifer developed fever and required systemic treatment.
with antibiotics and fluid therapy during the last month.

Persistent and marked leukocytosis was the characteristic feature of the hematological findings of the BLAD-affected heifer, as observed previously [8, 13]. In the analysis of serum proteins, persistent and hyper γ-globulinemia was evident, major parts of the γ-globulin was IgG, indicating that the production of immunoglobulins by B lymphocyte-plasma cell lineage is functioned well. Persistent hyper γ-globulinemia may have contributed to the required environment for the synthesis of amyloid [18, 20]. However, the exact mechanism by which this BM transplanted BLAD heifer synthesized the k-amyloid remains to be elucidated.

It is recognized that inflammatory cytokines, especially TNF-α and IL-1β contribute to the pathogenesis of acute injury in bovine respiratory diseases and alveolar macrophages are a major source of TNF-α and IL-1β in response to pathogens [21]. IL-1β contributes to the defence response against pathogens by promoting the infusion of leukocytes into sites of infection [2]. IL-1β-mediated up regulation of adhesion molecules results in an increase in neutrophil and lymphocyte adhesion on vascular endothelial cells [1, 15, 16]. The higher concentration of IL-1β in serum from the affected heifer may have contributed to the activation of the host defense mechanisms. IL-1 stimulates both IL-6 and
TNF-α, which participate in the inflammatory process. Several cytokines such as IL-8, GM-CSF and TNF-α appear to have important roles for producing the leukocytosis. The reason why the TNF-α concentration was not elevated remains unclear. Even in calves suffering from natural bronchopneumonia either in the acute phase or during convalescence, absence of circulating TNF-α had been detected by Espinasse et al. [5]. The number of leukocytes in peripheral blood in BLAD-calf ranged from 40 to 110 x 10^9/μl, and 80% of them were segmented neutrophils. These values were 10 to 20 times higher than those of normal controls [13]. It is likely that IL-8 is produced by neutrophils and is the potent factor for inducing neutrophilia. An increase in the level of plasma IL-8 is one of possible parameters in inflammatory conditions [9, 22]. To evaluate the possible cause of marked and persistent neutrophilia in this BLAD heifer, expressions of mRNA for IL-1β, IL-8 and GM-CSF on leukocytes from this BLAD heifer were determined. IL-8 and GM-CSF mRNA expression on leukocytes from this BLAD heifer were detected in the BM-treated heifer compared to controls. This finding suggests that hematopoiesis of leukocytes in bone marrow has been highly activated, and the production of neutrophils appears to be enhanced. The concentration of GM-CSF in serum from the affected heifer was not determined, and possible role of GM-CSF for the production of bovine neutrophils in bone marrow remains to be evaluated.

IL-6 is a potent cytokine to associate with the production of γ-globulin. In the analysis of the expression of mRNA for IL-6 in neutrophils, enhanced expression of IL-6 was detected in neutrophils from the BLAD heifer when compared to that of control neutrophils. Serum levels of IL-6 were increased in the BLAD heifer from 1.4 to 4 years old when compared to those of the BLAD heifer at 6 months old and normal controls, and they appeared to be associated with the increment of serum IgG. Increased levels of serum IL-6 found in the BLAD heifer may have enhanced differentiation of B-lymphocytes to plasma cells resulting in the persistent hyper γ-globulinemia. This also suggests that humoral immunity of the affected heifer is enhanced under the incapability of β2 integrin-mediated functions of phagocytic cells in the affected heifer.

In summary, the clinical condition of the affected heifer was relatively stable after BM-transplantation. Serum IL-1β and IL-6 concentrations in the affected heifer were evaluated in association with the increments of γ-globulinemia. Persistent hyper γ-globulinemia of the affected heifer was associated with elevated levels of IL-6 and its mRNA expression on leukocytes. The pathobiological profiles observed in the BLAD heifer demonstrated active humoral immunity in leukocyte β2 integrin deficiency.

ACKNOWLEDGEMENTS. The authors thank Dr. Gary J. Kociba, Department of Veterinary Biosciences, College of Veterinary Medicine, The Ohio State University, Columbus, Ohio, for critical reviewing of the manuscript. This study was supported in part by a Grant-in-Aid for Research Fund (No.11460146) from the Ministry of Education, Science and Culture, Japan, and by a Frontier Research Grant, Tokyo, Japan.

REFERENCES


生化学：
甲状腺ホルモンはラット褐色脂肪細胞の分化過程で作用してGLUT4の発現及びインスリンの受容性グルコース輸送系を増強する——志水耕一*1、鶴浜孝**1（愛媛大学医学部医化学教室、*現：岐阜大学農学部農獣医学教室）...677-681

ラットの新生仔ラットの脂肪組織から前駆細胞を分離し、T3非存在下あるいは存在下で分化させた。鈴田ペロリブリを用いて、グルコース輸送体(GLUT)の発現とインスリンにより生ずるラットの褐色脂肪組織に対する甲状腺ホルモン(T3)の作用を検討した。新生仔ラットの脂肪組織から前駆細胞を分離し、T3非存在下あるいは存在下で分化させた。ウエスタンプロット法による検討の結果、T3は処理効果にGLUT4の発現を増強することが明らかとなった。GLUT1発現は、T3の影響を受けなかった。T3処理により、インスリン刺激時の最大グルコース輸送速度の上昇に多大な影響を示され、インスリン感受性が高まることが明らかとなった。一方、NEによって促進されるグルコース輸送系は、T3処理によってほとんど影響を受けなかった。以上の結果から、T3は褐色脂肪細胞の分化過程に作用してGLUT4の発現及びインスリンの受容性グルコース輸送系を増強することが明らかとなった。

臨床病理学：
長期間生存した牛白血球粘着不全症牛の血清サイトカイン動態に関する検討——永幡聡**1、織部善男**1、村田公男**1、岩井雅史**1（岐阜大学農学部農獣医学教室、*現：岐阜大学農学部農獣医学教室）...683-687

骨髄移植を施した牛白血球粘着不全症(BLAD)牛のサイトカイン動態の検討を目的に、臨床ならびに臨床病理学的観察を移植後27カ月目から死亡までの19カ月間に渡って実施した。疾患牛の臨床状態は概ね良好に推移した。持続性γ-グロブリン血症と血清免疫グロブリンIgGの増高が続いて確認された。疾患牛の血清中GLUT和IGの濃度は15.8〜321.7ng/mlであり、最高値は血清中のGLUTのピークと一致した。血清GLUTは0.70〜27.9ng/mlであり、この増高は血清IGの増加にほぼ平行した動きを示すように見られた。GLUT1、GLUT4、GLUT6およびβグルカン、マクロファージコロニー刺激因子(M-CSF)の各mRNAは疾患牛の好中球において、対照牛のそれらに比較して増加していた。持続性高γ-グロブリンはGLUT4 mRNAの増加とその血清レベルの関連性が見られた。以上の結果は骨髄移植後の臨床経過の生存環境においては、血清のβ2-インターロイキン欠損状態において疾患の血液免疫機器の変化とともに、血清のサイトカイン活性ならびに好中球のサイトカイン発現の増高との関連性が示唆された。

胎盤微細毛細胞を用いたラット胎盤アミノ酸輸送活性に及ぼす上皮成長因子(EGF)の影響——松原孝一*1、枝野veal**1、木下隆**1（岐阜大学農学部農獣医学教室、*現：岐阜大学農学部農獣医学教室）...689-692

妊娠ラットに上皮成長因子(EGF)を、妊娠18日目から21日目まで、12時間おきに計6回投与(100および200μg/検体)した、妊娠21日目の胎盤から調製した胎盤微細毛細胞を用いて、アミノ酸のα-アミノ酸(ロイシン、プロリンおよびアルギニン)輸送活性に及ぼすEGFの影響について検討した。微細毛細胞へのα-アミノ酸依存性プロリンの最大取り込み量(Vmax)は、EGF投与により用量依存的に増加した。ロイシン、アルギニンの取り込みは用量依存的に増加した。これらは胎盤微細毛細胞のEGFとアルギニンの取り込みに変化は認められなかった。EGFを胎盤微細毛細胞のプロリン取り込みを増加させ、胎児血中のプロリン濃度を上昇させることで胎児発育に関与すると推測された。

動物行動学：
食肉目におけるドーパミン受容体D4遺伝子第3エクソン反復領域の塩基配列の比較(短報)——井上(村山)英松**1、北川英治**1（岐阜大学農学部）...747-749

NII-Electronic Library Service