Increased Activities of Cytosol Aminopeptidase and Lactate Dehydrogenase in Serum Originate from Lymphocytes in Necrotizing Lymphadenitis

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In three pediatric patients with necrotizing lymphadenitis, cytosol aminopeptidase activity (c-AP; EC 3.4.11.1) in serum was markedly increased to 508, 417, and 191 U/L, respectively (normal range 25–60 U/L). Lactate dehydrogenase (LD; EC 1.1.1.27) was also increased, with LD-3 predominating. The increased concentrations of c-AP and LD presumably originated from the destruction of infected, activated lymphocytes, especially T lymphocytes. Necrotizing lymphadenitis is probably caused by a lymphocytotropic virus.

Additional Keyphrases: pediatric chemistry • viruses • Kawasaki disease • bacterial lymphadenitis

We have recently reported that in patients with measles and rubella, viruses known to infect lymphocytes, the activity concentrations of cytosol aminopeptidase (c-AP; EC 3.4.11.1) and lactate dehydrogenase (LD; EC 1.1.1.27) in serum are markedly increased, and that these enzymes presumably originate from lymphocytes (1–3). Moreover, we have suggested (3) that increased c-AP and LD in serum are probably observed in other viral diseases that are known to be lymphocytotropic, such as human immunodeficiency virus.

Cervical lymphadenitis is common in children (4). We determined the activity concentrations of c-AP and LD in serum from children with cervical lymphadenitis. Marked increases in c-AP and LD were observed in some patients, who were clinically diagnosed as having necrotizing lymphadenitis (NL).

First identified in Japan, NL was subsequently recognized to have a worldwide distribution (5–12). The etiology of the lesion is still unknown, although it has been suggested that infection with Toxoplasma gondii (5) or Yersinia enterocolitica (9) might be involved, increases of the antibodies against these agents having been noticed in sera from some patients. However, these possibilities have not been confirmed by other workers.

Clinically, the lesion often appears as lymphadenopathy in the neck; the enlarged nodes are painful. Manifestation in other sites is less common (12). Fever and leukopenia are observed frequently, often with a transient rash (5). The prognosis is always excellent, however, with the lymphadenopathy resolving spontaneously, usually within two to three months.

Our purpose in this study was to measure serially activities of c-AP and LD in serum of patients with NL, to clarify the origin of the increase in these enzymes.

Subjects and Methods

Patients with NL

Diagnoses were based on the history, clinical findings, and laboratory results: persistent fever refractory to antimicrobial therapy, painful cervical lymphadenopathy, normal or low leukocyte count, normal or slightly increased values for C-reactive protein, and benign clinical course (5–12).

In all three patients, results of blood, throat, stool, and urine cultures done on admission were negative.

In all three patients, measurements of acute and convalescent titers of Epstein–Barr virus antibody (viral capsid antigen IgG and IgM, and Epstein–Barr nuclear antigen), cytomegalovirus antibody (IgG and IgM), and hemagglutination antibody for toxoplasma infection showed no significant changes. No antibody to human T lymphotropic virus type I was detected (13).

In all three patients, we measured acute and convalescent titers for complement fixation of influenza A and B, adenovirus, respiratory syncytial virus, and Mycoplasma pneumoniae, in addition to the hemagglutination inhibition titers of parainfluenza virus types 1 to 3, but were unable to demonstrate significant changes.

Patients with Bacterial Lymphadenitis and Kawasaki Disease

For comparison, we also measured some enzymes in serum of five patients with bacterial lymphadenitis and in seven with Kawasaki disease associated with marked lymphadenopathy.

For the patients with bacterial lymphadenitis, ages ranged from two to nine years (median age, six), and cultures were obtained by needle aspiration. Four of the five patients demonstrated Staphylococcus aureus, and in the other patient, group A hemolytic streptococcus was isolated. The patients with Kawasaki disease ranged in age from eight months to three years (median age, one year).

In all groups, we measured c-AP, LD, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (AP), and gamma-glutamyltransferase (GGT) at one- to five-day intervals. We also determined, at various times, LD isoenzymes in serum from the patients with NL.

Methods

c-AP was measured by the method described by Sugiyama et al. (14), in which L-leucinamide is used as the substrate. In this method, NADP⁺-dependent glutamate dehydrogenase from Proteus inconstans is linked, simultaneously detecting ammonia liberated by aminopeptidase activity. Liberated ammonia is measured continuously by the decreased absorbance at 340 nm caused by the oxida-
tion of NADPH (15).

The other enzymes were determined with a sequential multi-channel continuous-flow analyzer (Clinolyzer; Nippon Denshi Co., Tokyo, Japan) by well-established methods (16, 17).

Normal reference intervals are as follows: c-AP 25–60 U/L, LD 250–520 U/L, AST 20–50 U/L, and ALT 10–40 U/L. At our hospital, reference intervals for LD isoenzymes in normal children are as follows: LD-3 20.1–31.3%, LD-5 2.0–9.1%.

Cases with NL

Patient 1. A previously healthy seven-year-old boy had complained of painful lymphadenopathy and malaise since October 27, 1988, and developed a high temperature (39°C) on October 30. He was admitted to Nippon Kokan Hospital on November 9. Laboratory studies at the time of admission revealed a moderate increase in c-AP in serum, slightly increased value for C-reactive protein, and normal leukocyte count. A tender lymph node, about 1.5 cm in diameter, was palpable on the right side of his neck. The appearance of a chest roentgenogram was normal. Liver and spleen were not palpable.

Although he was treated initially with an antibiotic, cefotaxime, high fever persisted. On November 24, fever subsided, and lymphadenopathy regressed. He was discharged on November 29 and was completely well eight months after the onset of the disease.

Patient 2. A six-year-old boy was well until February 18, 1988, when high fever (38.5°C) and macular rash on the whole body appeared. He was admitted to Nippon Kokan Hospital on February 22. On admission, painful lymphadenopathy, about 1 cm in diameter, was present in his right neck. Laboratory studies showed a marked increase in c-AP in serum, slightly increased value for C-reactive protein, and moderate leukopenia. He was treated with cefotaxime. Although the rash gradually disappeared, fever persisted until February 29. Thereafter, lymphadenopathy rapidly regressed. He was discharged on March 5 and was in good health 16 months after discharge.

Patient 3. An eight-year-old girl had an infection of the upper respiratory tract and visited a hospital on October 11, 1987. At that time, enlargement of a lymph node in her left neck was noted for the first time. Thereafter, her temperature was occasionally above normal, about 38.5°C. She was admitted to Nippon Kokan Hospital on October 31 because of persistent fever, loss of appetite, and weight loss. On admission, a painful lymph node, about 1.5 cm in diameter, was palpable in her left neck. Admission laboratory studies revealed increased c-AP in serum, slightly increased value for C-reactive protein, and moderate leukopenia. She was treated with cefotaxime. However, fever persisted until November 7. Lymphadenopathy slowly regressed. She was discharged on November 11, and was completely well 15 months after discharge.

Results

Table 1 gives biochemical data on the three patients with NL. In patients 1 and 2, c-AP increased rapidly after admission, respectively peaking at 417 and 509 U/L, markedly increased values. Concurrently, LD increased, with the LD-3 isoenzyme predominating in the two patients. In patient 3, c-AP and LD activities were not as high as in patients 1 and 2, perhaps because she was admitted at a later stage of illness. AP and GGT activities were within the normal reference interval in all three patients.

Figure 1 illustrates sequential changes of c-AP and LD in patient 1. In this Figure, more data about these enzymes, not present in Table 1, are added. The c-AP activity increased from 103 to 232 U/L between November 9 and November 15, and LD activity went from 496 to 631 U/L, but ALT and LD-5 activities decreased (Table 1). Therefore, the increased activities of c-AP and LD cannot be interpreted as indicating liver dysfunction. They presumably originated from lymphocytes. Values for c-AP and LD peaked on November 21 at 417 and 801 U/L, respectively. LD-3 was increased (33.8% of total LD), whereas LD-5 was not changed (5.4%). Thus, at this stage, it seems that the increased activities of c-AP and LD in serum originated mainly from lymphocytes, although ALT activity increased to 140 U/L on November 24 (Table 1).

Similarly, in patients 2 and 3 with NL, increased activities of c-AP and LD in serum were presumably of lymphocyte origin.

Table 2 shows the mean peak values for c-AP and LD in patients with NL, bacterial lymphadenitis, and Kawasaki disease.

Table 1. Biochemical Data on Patients with Necrotizing Lymphadenitis

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<th>Patient</th>
<th>Date</th>
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<th>ALT</th>
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<th>LD</th>
<th>LD-3</th>
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N: not done.
Table 2. Comparison of NL, Bacterial Lymphadenitis, and Kawasaki Disease

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Discussion

Our data show that activity concentrations of c-AP and LD in serum are markedly increased in patients with NL, in contrast to those in patients with bacterial lymphadenitis and Kawasaki disease. Reportedly, activities of c-AP and LD in serum are increased in adult patients with NL (18), but the origin of the increased enzyme activities is unexplained.

C-AP preferentially hydrolyzes naturally occurring compounds such as L-leucinamide and L-leucylglycine, and it must be distinguished from microsomal aminopeptidase (EC 3.4.11.2) (15). C-AP is primarily located in the cytosol of liver cells, and its concentration in serum is thought to be a useful indicator of hepatocytic damage (15). It has been shown that c-AP is also contained in human lymphocytes (19) and that aminopeptidase activity may play an important role in the regulation of lymphocyte activation (20).

The enzyme LD is widely distributed among tissues in humans, but part of it has been shown to originate from human lymphocytes (21). Ringoir and Plum reported (22) that LD-3 was the most important lymphocyte fraction.

In our previous studies (1–3), we pointed out that with the appearance of rash in mesae and rubella, infected, activated lymphocytes are destroyed by an immunological reaction, causing the release of intracellular c-AP and LD.

We have also suggested that the activities of the two enzymes presumably reflect viral infectivity to lymphocytes, especially T lymphocytes. Moreover, intracellular enzymes such as c-AP and LD probably increase only when T lymphocytes are stimulated.

Based on the analogy with measles and rubella infection (1–3), it is probable that increased activities of c-AP and LD in patients with NL reflect the destruction of activated T lymphocytes. Although the etiology of NL is still unknown, our patients' biochemical data suggest that NL may be caused by infection with a lymphocytotropic virus, especially a virus infecting T lymphocytes, such as human T lymphocytotropic virus type I (13) and human herpesvirus-6 (23). Human T lymphocytotropic virus type I may not be the causative agent for NL, according to the serological data from our patients.

In NL, the main changes are reported to occur in T-cell zones of the lymph node; histologically, the affected lymph node shows focal cortical or paracortical proliferation of histiocytes, reticulum cells, or transformed lymphocytes, associated with complete or incomplete necrosis of lymphoid tissue (5–12). This pathological finding supports our hypothesis that increases in c-AP and LD in serum in NL are derived from the destruction of infected, activated T lymphocytes.

In conclusion, from a biochemical standpoint, NL appears to be caused by a lymphocytotropic virus, particularly one that infects T lymphocytes.

We acknowledge with thanks the excellent technical assistance of Mr. S. Hiyoshi and the other members of the clinical chemistry laboratory at Nippon Kokan Hospital.

References