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Reduced streptozotocin-induced insulitis in CD-1 mice by treatment with anti-intercellular adhesion molecule-1 and anti-lymphocyte function associated antigen-1 monoclonal antibodies together with lactic dehydrogenase virus infection

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Summary. Multiple low-dose streptozotocin (SZ)-induced insulitis is an animal model for insulin-dependent diabetes mellitus characterized by a mononuclear cell infiltration. SZ-induced insulitis and blood glucose concentrations were reduced by treatment with anti-intercellular adhesion molecule-1(ICAM-1) and anti-lymphocyte function associated antigen-1 (LFA-1) monoclonal antibodies. This suppressing effect was also seen in mice infected with lactic dehydrogenase virus (LDV). These results suggest that the expression of ICAM-1 in islets and LFA-1 on mononuclear cells may be important in the development of SZ-induced insulitis. The suppressive effect of LDV infection on the development of insulitis is discussed.

Keywords: insulitis, LDV, SZ, ICAM-1, LFA-1

Multiple low-dose streptozotocin(SZ)-induced insulitis in mice is considered as a model of insulin-dependent diabetes mellitus (IDDM) (Like & Rossini 1976). It may be the result of cell-mediated immunity, because mononuclear cell infiltration, such as macrophages and lymphocytes, are seen in and around islets (Nakamura *et al.* 1984). Also SZ-induced insulitis is inhibited in mice treated with anti-T cell antibody (Herold *et al.* 1987) or silica (Oshilewshik *et al.* 1985). These data suggest that immunologic injury by these cells may cause damage to islets. A number of accessory molecules that cooperate with specific receptors and play an important role in the cell interactions of the immune system have been defined. One of these, intercellular adhesion molecule-1(ICAM-1), which is a ligand for lymphocyte function associated antigen-1(LFA-1), arrests circulating leucocytes, which is the first step in their recruitment to inflammatory sites (Osborn *et al.* 1989). Furthermore, it has recently been reported that ICAM-1 can be induced on isolated mouse pancreatic β cells by inflammatory cytokines (Prieto *et al.* 1992).

On the other hand, lactic dehydrogenase virus (LDV) infection in mice causes modulation of inflammatory and immune reactions without pathological changes (Rowson & Mahy 1985). The host is mouse and the target cells are a subpopulation of macrophages (Rowson & Mahy 1985). LDV infection causes suppression of delayed type hypersensitivity (Hayashi *et al.* 1991b), inhibited expression of Ia antigen on macrophages and antigen-presenting capacity of macrophages (Inada & Mims 1985a, b; Isakov *et al.* 1982), and chemotactic activity of macrophages (Stevenson *et al.* 1980). Also, LDV infection prevents autoimmune reaction and autoimmune diseases in mice (Hayashi *et al.* 1992; 1993; Inada & Mims 1986b; Oldstone & Dixon, 1972; Takei *et al.* 1992). The present study investigated the effect of anti-ICAM-1 and

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anti-LFA-1 monoclonal antibodies together with LDV infection on SZ-induced insulitis in ICR (CD-1) mice.

Materials and methods

Animals

Eight-week-old male ICR (CD-1) mice were obtained from SLC Co. (Shizuoka, Japan). Autoclaved pellets MF (Oriental Yeast, Todyo, Japan) and tap water were supplied *ad libitum*. Each set of experiments comprised five or 11 mice.

Virus

A stock preparation of LDV (Hayashi *et al.* 1988) kindly supplied by Dr A. L. Notkins (National Institute of Dental Research, National Institutes of Health, USA) was used throughout the experiments. Mice were infected with virus at the age of 9 weeks by intraperitoneal injection of $10^{4.5}$ median infectious dose. Mice were infected with LDV just before the first SZ injection (this day is referred to as Day 0).

Experimental procedures

Streptozotocin (SZ; Sigma, St Louis, MO, USA) was dissolved in citrate buffer (pH 4.0 ~ 4.5) before use. The injection dose was adjusted to 40 mg in 0.2 ml citrate buffer per kg body-weight. Groups of mice with or without LDV infection were injected intraperitoneally with SZ daily for 5 days (from Day 0 to Day 4). SZ-treated mice with or without LDV-infection were given intraperitoneal injections of 50 μg of rat monoclonal antibody (IgG2a) against mouse ICAM-1 (Seikagagu, Tokyo, Japan) and 50 μg of rat monoclonal antibody (IgG2b) against mouse LFA-1 (CDIIa; Pharmingen, CA, USA) in 0.5 ml PBS 7 days after the first treatment (Day 0) with SZ, since the development of insulitis begins 7 days after first treatment with SZ (unpublished data). SZ-treated mice with or without LDV-infection were also given intraperitoneal injections of 0.5 ml PBS as control. Glucose levels in the blood from non-fasting mice were examined at −1, 6, 12 and 15 days after first SZ treatment. Blood specimens were obtained by retro-orbital bleeding with heparinized micropipettes, and plasma glucose concentrations were measured by the glucose oxidase method.

Pancreata were fixed in Bouin's solution and sections were stained with haematoxylin and eosin (HE) and aldehyde–fuchsin. Histopathology was evaluated 15 days after the first SZ treatment. The severity of the mononuclear cell infiltration was evaluated by light microscopy, and an insulitis score was designated for each islet according to the following scale: 0, no insulitis; 1, peri-insulitis; 2, insulitis < 25% of islet area; 3, insulitis 25–50% of islet area; and 4, insulitis > 50% of islet area. The grade of insulitis in a mouse was expressed as the average score calculated by the following equation: score index = total score/number of islets. About 20 different islets were examined per pancreas.

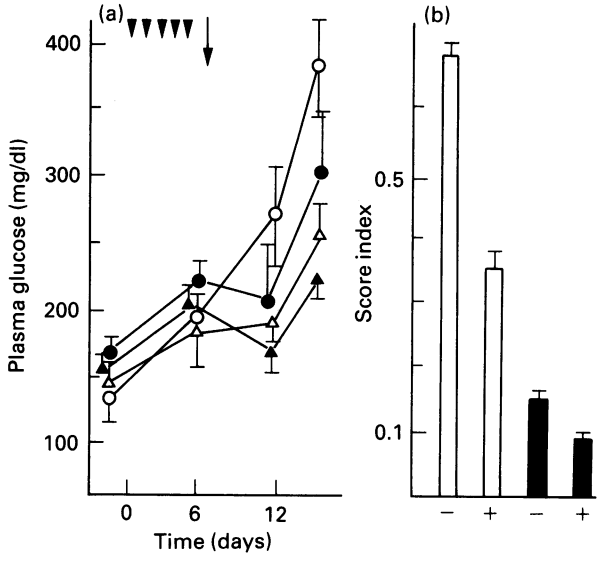

Figure 1. a, Glucose concentrations in mice treated with SZ under the condition with or without LDV infection. Mice were infected with LDV at the same time as the first treatment day of SZ(Day 0). Glucose concentrations were determined before and after treatment of SZ. Mice were treated with SZ for 5 days (arrow heads). The arrow indicates the injection of antibodies. ○, Uninfected ($n = 11$); ●, uninfected plus antibodies ($n = 5$); △, LDV-infected ($n = 11$); ▲, LDV-infected plus antibodies ($n = 5$). Each point represents the mean ± s.e. b, Score index in SZ treated mice +, with or −, without antibodies under the condition ■, with or □, without LDV-infection. Score index was examined 15 days after first treatment of SZ. Each point represents the mean ± s.e.

Statistics

In all experiments data points are expressed as the mean ± standard error (s.e.). Differences between means with P values less than 0.05 ($P < 0.05$) were considered to be significant.

Results

Effect of monoclonal antibodies against ICAM-1 and LFA-1 on plasma glucose concentrations and score index (severity of insulitis)

Plasma glucose concentrations in LDV-infected and uninfected mice without SZ were about 150 mg/dl during the experimental periods (data not shown). As shown in

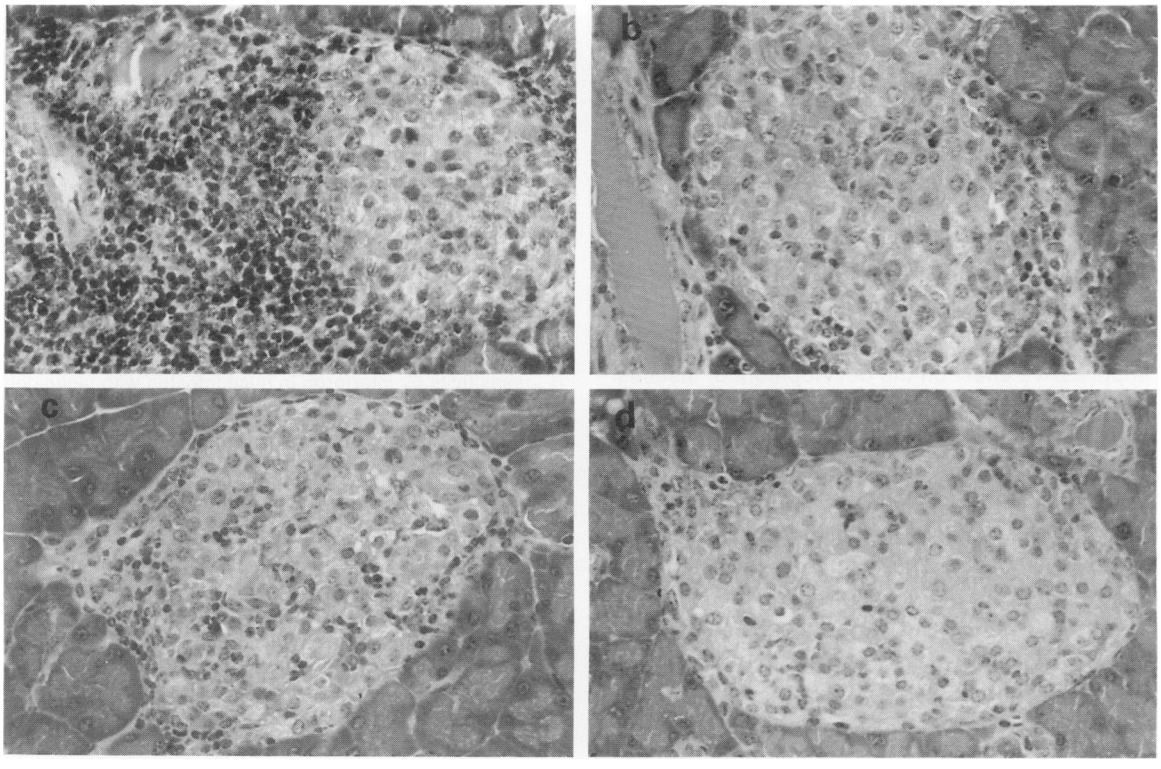

Figure 2. Pancreatic islets of a, uninfected SZ-treated mice and b, uninfected SZ-treated mice with antibodies. Pancreatic islets of c, LDV-infected SZ-treated mice and d, LDV-infected SZ-treated mice with antibodies. HE. × 110.

Figure 1a, glucose concentrations in uninfected SZ-treated mice increased to over 250 mg/dl at 12 days and about 400 mg/dl 15 days after the first SZ treatment (Day 0). However, in SZ-treated uninfected mice with antibody treatment glucose concentrations were lower than those in uninfected mice treated with SZ alone ($P < 0.05$ at 15 days). As shown in Figure 1a, in LDV-infected mice glucose concentrations induced by SZ were lower than those in uninfected SZ-treated mice with or without antibodies ($P < 0.01$ at 12 and 15 days compared with uninfected SZ-treated mice). In SZ-treated LDV-infected mice glucose concentrations rose slightly above normal values when they were treated with antibodies. There were differences between SZ-treated LDV-infected mice and SZ-treated LDV-infected mice with antibodies ($P < 0.05$ at 15 days).

As shown in Figure 1b, in uninfected SZ-treated mice the antibodies reduced the score index from 0.67 to 0.35 (about 50% reduction, $P < 0.01$). The score index in infected SZ-treated mice was prominently suppressed ($P < 0.01$, compared to uninfected SZ-treated mice with or without antibodies). Furthermore the antibodies reduced the score index from 0.16 to 0.1 (about 40% reduction, $P < 0.05$) in infected mice. No insulitis was seen in uninfected or LDV-infected groups without SZ (data not shown).

Though the severity of the inflammatory changes varied, in general extensive necrosis of islets with severe mononuclear cell infiltration was seen in and around islets in SZ-treated uninfected mice (Figure 2a). On the other hand, a little islet cell necrosis with mild infiltration of mononuclear cells in (Figure 2b) and around islets, and in general predominantly peri-islet infiltration, were seen in uninfected SZ-treated mice with antibodies. In contrast, a few necrotic cells with mild mononuclear cell infiltration were seen in (Figure 2c) and around islets in SZ-treated mice with LDV-infection. A few necrotic cells and cell infiltrates were seen in islets in LDV-infected mice treated with antibodies (Figure 2d).

Discussion

The present study showed that SZ-induced insulitis, which is characterized by mononuclear cell infiltration of lymphocytes and macrophages, was reduced in CD-1 mice by the treatment of antibodies directed against ICAM-1 and LFA-1. These results suggest that ICAM-1 expression on the endothelium (Patarroyo 1991), and β cells in the islets (Prieto *et al.* 1992) may be responsible

for development of insulitis in SZ-treated mice. Also, LFA-1 expressing on T lymphocytes (Dustin & Springer 1988) and macrophages (Patarroyo 1991) may be important for insulitis, since macrophage and T-cell mediated β cell destruction has been suggested (Hutchings *et al.* 1990). In addition it has been reported that an adhesion-blocking antibody against CD11b/CD18, a β_2 integrin expressed mainly by myelomonocytic cells, reduces incidence of IDDM in non-obese diabetic mice (Hutchings *et al.* 1990).

As reported here, LDV infection also exerted a prominent suppressive effect on the development of insulitis. There are several possible explanations for this. It has been reported that isolated β cells of the pancreas (Prieto *et al.* 1992) and vascular endothelium (Pober *et al.* 1986) express ICAM-1 following stimulation with inflammatory cytokines, such as γ-interferon, tumour necrosis factor-α and interleukin-1 (IL-1). Thus suppressed insulitis by LDV-infection may be due to suppressed production of these cytokines. As previously reported in LDV-infected mice, IL-1 production was suppressed (Hayahsi *et al.* 1991a). Production of other cytokines may thus be suppressed in LDV-infected mice. Further studies are needed to clarify these points. Furthermore, reduced macrophage chemotaxis (Stevenson *et al.* 1980) may be responsible for reduced insulitis in LDV-infected mice, since macrophage infiltration precedes (Kolb-Bachofen *et al.* 1988) and is a prerequisite for lymphocytic insulitis in pancreatic islets of the diabetic prone BB rat (Haneberg *et al.* 1989). The levels of macrophage specific chemotactic lipid released from islet by in-vivo SZ administration (Muir *et al.* 1991) may be negligible, since there are no differences in direct islet cell damage mediated by SZ between uninfected and LDV-infected mice in the preinsulitis phase (unpublished observation). Reduced antigen presenting activity and induction of suppressor T cells in LDV-infected mice (Inada & Mims 1986a; Isakov *et al.* 1982) may be other factors for reduction of insulitis in LDV-infected mice.

As reported here, surprisingly only one injection and small doses of antibodies reduced the immune mediated insulitis. Our observations indicate a potential therapeutic application of these antibodies. We further suggest that monoclonal antibody directed against specific intercellular adhesion molecules and the corresponding ligands may be effective in the treatment of certain autoimmune diseases, and that the therapeutic benefit may be achieved without provoking an undesirable, and potentially hazardous, host immune response.

References

Dustin M.L. & Springer T.A. (1988) Lymphocyte function-associated antigen-1 (LFA-1) is one of at least 3 mechanisms for T lymphocyte adhesion to cultured endothelial cells. *J. Cell Biol.* **107,** 321–331.

Haneberg H., Kolb-Bachofen V. & Kantwerk-Funke G. (1989) Macrophage infiltration precedes and is prerequisite for lymphocytic insulitis in pancreatic islets of pre-diabetic BB rat. *Diabetologia* **32,** 126–134.

Hayashi T., Iwata H., Ozaki M., Yamamoto H. & Onodera T. (1991a) Decrease in neutrophil migration induced by endotoxin and suppression of interleukin-1 production by macropages in lactic dehydrogenase virus-infected mice. *J. Comp. Path.* **104,** 161–170.

Hayahsi T., Koike T., Hasegawa T., Tsurudome M., Ozaki M., Yamamoto H. & Onodera T (1991b) Inhibition of contact sensitivity by interferon in mice infected with lactic dehydrogenase virus. *J. Comp. Path.* **104,** 357–366.

Hayashi T., Mori I. & Yamamoto H. (1992) Lactic dehydrogenase virus infection prevents development of anti-nuclear antibody in (NZB × NZW)F_1 mice; role of prostaglandin E_2 and macrophage Ia antigen expression. *In. J. Exp. Path.* **73,** 593–601.

Hayashi T., Noguchi T. & Kameyama Y. (1993) Suppression of development of anti-nuclear antibody and glomerulonephritis in NZB × $NZWF_1$ mice by persistent infection with lactic dehydrogenase virus: possible involvement of superoxide anion as a progressive effector. *Int. J. Exp. Path.* **74,** 553–560.

Hayashi T., Salata K., Kingman A. & Notkins A.L. (1988) Regulation of enzyme levels in the blood: influence of environmental and genetic factors on enzyme clearance. *Am. J. Path.* **132,** 50–510.

Herold K.C., Nontag A.G. & Fitci F.W. (1987) Treatment with anti-T lymphocyte antibodies prevents induction of insulitis in mice given multiple dose of streptozotocin. *Diabetes* **36,** 796–801.

Hutchings P.R., Rosen H., O'Relly L., Simpson E., Gordon S. & Cooke A. (1990) Transfer of diabetes in mice prevented by blockade of adhesion-promoting receptor on macrophages. *Nature* **348,** 639–642.

Inada T. & Mims C.A. (1985a) Mouse Ia antigens are receptors for lactate dehydrogenase virus. *Nature* **309,** 59–61.

Inada T. & Mims C.A. (1985b) Pattern of infection and selective loss of Ia positive cells in suckling and adult mice inoculated with lactic dehydrogenase virus. *Arch. Virol.* **86,** 151–165.

Inada T. & Mims C.A. (1986a) Live lactic dehydrogenase-elevating virus (LDV) induces suppressor T cells that inhibit the development of delayed hypersensitivity to LDV. *J. Gen. Virol.* **67,** 2106–2112.

Inada T. & Mims C.A. (1986b) Infection of mice with lactic dehydrogenase virus prevents development of experimental allergic encephalomyelitis. *Neuroimmunol.* **11,** 53–56.

Isakov N., Feldmann M. & Segal S. (1982) Acute infection of mice with lactic dehydrogenase virus (LDV) impairs the antigen-presenting capacity of their macrophages. *Cell. Immunol.* **66,** 317–332.

Kolb-Bachofen V., Epstein S., Kiesel V. & Kolb H. (1988) Low-dose streptozotocin-induced diabetes in mice. Electron microscopy reveals single-cell insulitis before diabetes onset. *Diabetes* **37,** 21–27.

Like A.A. & Rossini A.A. (1976) Streptozotocin-induced pancreatic insulitis: New model of diabetes mellitus. *Science* **193,** 415–417.

Muir A., Rovin B.H., Lacy Pe & Schreiner G.F. (1991) Macrophage-specific chemotactic lipid release by in vivo streptozotocin-administered mouse islets. *Diabetes* **40,** 1459–1466.

Nakamura M., Nagajuchi S., Yamaguchi K. & Takaki R. (1984) The

role of thymic immunity and insulitis in the development of streptozotocin-induced diabetes in mice. *Diabetes* **33,** 894–900.

OLDSTONE M.B.A. & DIXON F.J. (1972) Inhibition of antibodies to nuclear antigen and to DNA in New Zealand mice infected with lactate dehydrogenase virus. *Science* **175,** 784–786.

OSBORN L., HESSIONO C., TIZARD R., VASSALLO C., LUHOWSHYJ S., CHI-ROSSO G. & LOBB R. (1989) Direct expression cloning of vascular cell adhesion molecule 1, a cytokine-induced endothelial protein that binds to lymphocytes. *Cell* **259,** 1203–1211.

OSHILEWSHIK V., KISESL B. & KOLB H. (1985) Administration of silica prevents diabetes in BB-rats. *Diabetes* **34,** 197–199.

PATARROYO M. (1991) Leukocyte adhesion in host defense and tissue injury. *Clin. Immunol. Immunopath.* **60,** 333–348.

POBER J.S., GIMBRONE M.A., LAPIERRE L.A., MENDRICK D.L., FIERS W., ROTHLEIN R. & SPRINGER J.A. (1986) Overlapping patterns of activation of human endothelial cells by interleukin 1, tumor necrosis factor, and immune interferon. *J. Immunol.* **137,** 1893–1896.

PRIETO J., KAAYA E.E., JUNTTI-BERGGREN L., BERGGREN P-O., SANDLER S., BIBERFELD P. & PATARROYO M. (1992) Induction of intercellular adhesion molecule-1 (CD54) on isolated mouse pancreatic β cells by inflammatory cytokines. *Clin. Immunol. Immunopath.* **65,** 247–253.

ROWSON K.E.K. & MAHY B.W.J. (1985) Lactate dehydrogenase-elevating virus. *J. Gen. Virol.* **66,** 2297–2312.

STEVENSON M.M., REES J.C. & MELTZER M.S. (1980) Macrophage function in tumor bearing mice: Evidence of LDV-associated changes. *J. Immunol.* **124,** 2892–2899.

TAKEI I., ASABA Y., KASATANI T., MARUYAMA T., WATANABE K., YANAGAWA T., SARUTA T. & ISII T. (1992) Suppression of development of diabetes in NOD mice by lactate dehydrogenase virus infection. *J. Autoimmun.* **5,** 665–673.