Augmented lipopolysaccharide-induction of the histamine-forming enzyme in streptozotocin-induced diabetic mice

Senri Oguria,b, Katsutoshi Motegia, Yasuo Endob,*

a Department of Oral and Maxillofacial Surgery, Graduate School of Dentistry, Tohoku University, 4-1 Seiryo-machi, Aoba-ku, Sendai 980-8575, Japan
b Department of Pharmacology, Graduate School of Dentistry, Tohoku University, 4-1 Seiryo-machi, Aoba-ku, Sendai 980-8575, Japan

Received 18 July 2002; received in revised form 30 October 2002; accepted 13 November 2002

Abstract

Disorders of the microcirculation and reduced resistance to infection are major complications in diabetes. Histamine enhances capillary permeability, and may also reduce cellular immunity. Here we demonstrate that streptozotocin (STZ)-induced diabetes in mice not only enhances the activity of the histamine-forming enzyme, histidine decarboxylase (HDC), but also augments the lipopolysaccharide (LPS)-induced elevation of HDC activity in various tissues, resulting in a production of histamine. The augmentation of HDC activity occurred as early as 2 days after STZ injection, but was not seen in nondiabetic mice. When given to STZ-treated mice, nicotinamide, an inhibitor of poly(ADP-ribose) synthetase, reduced both the elevation of blood glucose and the elevations of HDC activity and histamine production. These results suggest that hyperglycemia may initiate a sequence of events leading not only to an enhancement of basal HDC activity, but also to a sensitization of mice to the HDC-inducing action of LPS. We hypothesize that bacterial infections and diabetic complications may mutually exacerbate one another because both involved an induction of HDC.

© 2002 Elsevier Science B.V. All rights reserved.

Keywords: Histamine; Histidine decarboxylase; Diabetes; Hyperglycemia; Periodontitis; Infectious disease

1. Introduction

Disorders of the microcirculation are major complications in both insulin-dependent diabetes mellitus (IDDM) and non-insulin-dependent diabetes mellitus (NIDDM). Histamine is a potent stimulator of capillary permeability, and there are reports indicating that antihistamines can reverse the breakdown of the blood–ocular barrier seen in STZ-induced diabetic rats [1] and also reduce the blood–retinal barrier permeability in IDDM patients with non-proliferative retinopathy [2]. Histamine can also reduce the expression of the tight-junction protein ZO-1 in retinal endothelial-cell culture [3]. Moreover, histamine may activate osteoclasts [4,5], and osteoporosis is another complication seen in diabetic patients. These findings strongly suggest that an enhanced synthesis of histamine is involved in the disorders, particularly of the microcirculation, that are associated with diabetes.

In addition to the disorders described above, diabetes is thought to be an important factor exacerbating infectious diseases or sensitizing patients to infection [6]. For example, recent studies lead to the consensus that gram-negative bacteria such as Prevotella intermedia and P. gingivalis are important causes of periodontitis [7,8], and that diabetic patients have an increased risk of this condition [9]. Indeed, it has been suggested that diabetes may exacerbate periodontitis [10]. Conversely, it has also been suggested that periodontitis may exacerbate diabetic complications [11]. Interestingly, evidence is accumulating to suggest that histamine reduces cell-mediated immunity [12,13] because it inhibits the production of Th1-cytokines (such as IFN-γ, IL-2 and IL-12) [14–16] and stimulates the production of Th2-cytokines (such as IL-6 and IL-10) [15,17,18]. Indeed, histamine has been suggested to prolong or exacerbate infectious diseases [19].

It is widely known that histamine is released from mast cells or basophils in response to antigens. However, histamine can also be released from other types of cells in which it is newly formed through the induction of the histamine-forming enzyme, histidine decarboxylase (HDC) [20–23].
The LPS obtained from *Escherichia coli* and *P. intermedia* (the latter being a prevalent bacterium in both periodontitis and endodontal infections) are potent inducers of HDC in various tissues in mice, including the mandible [21,24–26]. Peptidoglycan, a cell-wall component of gram-positive bacteria, can also induce HDC in the same tissues in which LPS induces HDC [27]. The induction of HDC by LPS occurs through the de novo formation of HDC-mRNA [28], and we have suggested that HDC may be induced in vascular endothelial cells by LPS [29]. Unlike mast-cell histamine, the newly formed histamine is released immediately from the cells in which it is produced [20,22–24,30].

In the present study, having considered the background described above, we examined the effect of the hyperglycemia induced in mice by STZ on the elevation of HDC activity induced by an LPS from *E. coli* or *P. intermedia*.

### 2. Materials and methods

#### 2.1. Animals and materials

Male BALB/c mice (5 weeks old) were obtained from the facility for experimental animals in Tohoku University. They were kept under standard conditions [a light (07:00–19:00)–dark (19:00–07:00) cycle at a controlled temperature (23 ± 1 °C)] for 7–10 days, and given standard food pellets (“LabMR Stock”; Nihon Nofsan Inc., Yokohama, Japan) and water ad libitum. Streptozotocin (STZ), nicotinamide (NA), and N-acetylcysteine-HCl (NAC) were purchased from Sigma (St. Louis, MO, USA). LPS from *E. coli* O55:B5 prepared by Boivin’s method was obtained from Difco Laboratories (Detroit, MI, USA). LPS from *P. intermedia* ATCC 25611, which was prepared by the method of Galanos et al. [31] (phenol–chloroform–petroleum ether extraction), was kindly provided by Dr. H. Takada [32]. Its properties and purity have been described in previous reports [33,34]. All experiments conformed to national requirements (Japanese law no. 105, notification no. 6) and complied with the Guidelines for Care and Use of Laboratory Animals in Tohoku University.

#### 2.2. Injection of STZ, NA, NAC, or LPS

Although in many studies, STZ is dissolved in buffers containing citric acid (pH 4–5), citric acid itself has various biological effects, such as on the metabolism of Fe³⁺, sugars, and fatty acids. Therefore, in the present study, STZ was dissolved in sterile saline and injected intravenously within 30 min of its preparation, and we confirmed that injections of STZ (200 mg/kg) in this way produced a hyperglycemia equal to that produced by STZ dissolved in citrate buffer. Mice were starved for 8–10 h before STZ injection, and STZ was injected at 17:00 to 19:00.

NA, NAC, and LPS were dissolved in sterile saline and injected intraperitoneally (0.1 ml per 10 g body weight). NA or NAC, each at 500 mg/kg, was injected at 1 h before STZ injection. At this dose, NA inhibits the diabetic action of STZ [35], and NAC markedly reduces the induction of NO synthase by alloxan [36] and has been suggested to scavenge reactive oxygen species in mice [37].

#### 2.3. Determination of blood glucose

A segment of tail vein was pierced by a needle and the blood extruded (about 5 μl) was directly applied onto a strip for the determination of glucose (based on a glucose-dehydrogenase method) using a glucometer (Accu-Chek Advantage; Roche Diagnostics K.K., Tokyo, Japan). The levels of blood glucose were expressed as mg/dl.

#### 2.4. Assay for HDC activity

HDC activity was assayed using our previously described method [38] with a slight modification [39]. Briefly, mice (anesthetized by ether) were decapitated and the tissues rapidly removed and stored in a jar with dry ice. The soft tissues covering the mandible were wiped off with soft paper, and the incisor teeth and molars were extracted from the mandible using tweezers. After each tissue sample (less than 250 mg) had been put into a cooled Teflon tube with phosphorylated cellulose and 2.5 ml of ice-cold 0.02 M phosphate buffer (pH 6.2) containing pyridoxal 5'-phosphate and dithiothreitol, it was homogenized. The supernatant obtained after centrifugation of the homogenate was used as the enzyme solution. The histamine in the tissues was bound to the phosphorylated cellulose, and was removed almost completely from the enzyme solution by the centrifugation. Reaction mixture (1 ml) containing the enzyme solution was incubated at 37 °C for 14 h with histidine. After the enzyme reaction had been terminated by adding HClO₄, the histamine formed during the incubation was separated by chromatography on a small phosphorylated cellulose column, then quantified fluorometrically as previously described [38]. HDC activity was expressed as nanomoles of histamine formed during a 1-h period of incubation by the enzyme contained in 1 g (wet weight) of each tissue (nmol/h/g). HDC activity in the bone marrow was expressed as the activity in 1 g (wet weight) of tibia, because this was assayed without separating the bone marrow [40].

#### 2.5. Determination of histamine

Histamine was extracted from tissues using 0.4 M HClO₄, separated by chromatography and determined fluorometrically, as previously described [38]. Histamine levels were expressed as nmol/g wet tissue.
2.6. Data analysis

Experimental values are given as mean ± standard deviation (S.D.). The statistical significance of the difference between two means was evaluated using a Student’s unpaired t-test, with P values less than 0.05 being considered significant.

3. Results

3.1. Elevation of HDC activity in various tissues of STZ-diabetic mice

Blood glucose was markedly elevated (to 300 mg/dl or more, see Fig. 4) at 7 days after an injection of STZ at a dose of 200 mg/kg. In all tissues examined from these diabetic mice, there was a significant elevation of basal levels of HDC activity at this time (Fig. 1).

In most of the subsequent experiments, we examined HDC activity in only a few of the tissues and organs shown in Fig. 1, such as organs rich in capillaries (liver or lung) or hematopoietic organs (spleen or mandible), because measuring HDC activity is very laborious, and because HDC has been suggested to be induced mainly in vascular endothelial cells and in hematopoietic precursor cells [29].

3.2. Augmentation of LPS-induced elevation of HDC activity in STZ-diabetic mice

Injection of E. coli LPS enhances the maximal HDC activity in various tissues in mice at 3–5 h after its injection [24]. Consequently, we tested HDC activity at 4 h after an LPS injection. As shown in Fig. 2, E. coli LPS (0.1 mg/kg) markedly elevated HDC activity, compared with the level seen in control mice (saline-injected). In this experiment, as well as in that illustrated by Fig. 1, a significant elevation of basal HDC activity was observed in STZ-diabetic mice. In diabetic mice at 7 days after STZ injection, the LPS-induced elevation of HDC activity was markedly augmented in all the tissues examined (liver, lung, spleen, and mandible). This augmentation was not detectable in nondiabetic (i.e., nonhyperglycemic) mice that had been given a lower dose of STZ, 100 mg/kg (data not shown).

Like E. coli LPS, P. intermedia LPS enhances the maximal HDC activity in various tissues in mice at 3–5 h after its injection [26]. When P. intermedia LPS (1 mg/kg) was injected into STZ-diabetic mice (Fig. 3), there was an augmentation of HDC induction, as there was with 0.1 mg/kg of E. coli LPS (Fig. 2), although the elevated levels of HDC activity were smaller with the former than with the latter (by about one half).
3.3. Effect of interval between STZ injection and LPS injection on the augmentation of the LPS-induced HDC elevation

Next, we tested how soon an augmentation of the LPS-induced HDC elevation can be detected following STZ injection. In this experiment, HDC activity in the liver was examined because the augmentation was large in this organ (Fig. 2). STZ (200 mg/kg) induced hyperglycaemia as early as 2 days after its injection (Fig. 4), but elevation of HDC activity by STZ itself was not significant at this time (data not shown). As shown in Fig. 4, a significant augmentation occurred when LPS was given as early as 2 days after an STZ injection. Fig. 4 also shows that the magnitude of the augmentation roughly paralleled that of the blood glucose elevation when the interval between the STZ and LPS injections was 2–7 days.

3.4. Effects of NA and NAC on blood glucose and on the augmentation by STZ of the LPS-induced HDC elevation

As described below (see Discussion), STZ has been shown to damage DNA either by alkylation or by the generation of oxygen radicals, leading to the death of islet β-cells and a consequent hyperglycaemia. Thus, for the mediator of the augmentation of the LPS-induced HDC elevation, there are two candidates, (i) hyperglycaemia itself (or disturbance of homeostasis due to the hyperglycaemia), or (ii) reactive oxygen species, but not hyperglycaemia. So, we carried out experiments to see which of these might be the more likely.

First, to test possibility (i), the effect of NA was examined. NA is thought to prevent the death of islet β-cells by maintaining NAD levels via an inhibition of poly(ADP-ribose) synthetase [41–43]. The elevation of blood glucose by STZ was largely (although not completely) prevented by NA, and the elevations of basal HDC activity in the liver and lung were either significantly reduced or showed a tendency to be reduced (Fig. 5). NA also largely prevented the augmentation of LPS-induced HDC activity seen in STZ-diabetic mice, i.e., HDC activity levels in the NA + STZ → LPS group were similar to those seen with LPS itself (see Fig. 2). In another experiment, however, NA was found not to reduce the HDC elevations induced by LPS alone in liver and lungs (data not shown).

Next, we examined the effect of NAC, a potent scavenger of reactive oxygen species. However, neither the elevation in blood glucose nor the elevation of HDC activity induced by STZ (150 and 200 mg/kg) alone was suppressed by NAC, nor was the HDC augmentation induced by a combination of STZ and LPS (data not shown).

3.5. Effects of STZ, LPS, and NA on histamine levels

Finally, we tested the effects of STZ, LPS, and NA on histamine levels (Fig. 6). *E. coli* LPS itself significantly increased histamine levels in the liver and lung and tended to increase it in the spleen. STZ by itself induced a similar pattern of changes. Injection of LPS into STZ-diabetic mice produced the highest levels of histamine seen in these three organs and in the blood. NA pretreatment significantly reduced the histamine increase induced in the liver by STZ alone. NA also significantly reduced the histamine increase induced by a combination of STZ and LPS in the three tissues tested and in the blood. However, in another experiment, NA had no significant effects on the histamine levels.

![Fig. 4. Effect of interval between STZ injection and LPS injection on augmentation by STZ of LPS-induced HDC elevation in the liver. Mice were injected with LPS (0.1 mg/kg) at 0, 2, 4, or 7 days after injection of STZ (200 mg/kg), and livers were removed 4 h after the injection of LPS. The values at time 0 show the HDC activity induced by LPS in STZ-non-treated mice. Blood glucose was measured 30 min before the LPS injection. Each value is mean ± S.D. from four mice. *P<0.05, **P<0.01, and ***P<0.001 vs. time 0.](image)

![Fig. 5. Effects of NA on blood glucose and on the augmentation by STZ of LPS-induced HDC elevation in the lung. NA (500 mg/kg) was injected intraperitoneally at 1 h before STZ injection (200 mg/kg), and LPS was injected 7 days later. Saline (S) was injected into other groups at various injection times instead of NA or LPS. Blood glucose was measured 30 min before the final injection. Livers and lungs were removed 4 h after the final injection. Each value is mean ± S.D. from four mice. *P<0.05, **P<0.01, ***P<0.001 vs. S + S → S. #P<0.05, ##P<0.01, ###P<0.001 between the two groups indicated.](image)
increases induced by LPS alone in the liver, lung, spleen, and blood (data not shown).

4. Discussion

We can summarize the results of the present study as follows: (i) at 7 days after an intravenous injection of STZ, there was a modest, but significant enhancement of basal levels of HDC activity in a number of tissues, (ii) the elevation of HDC activity induced in various tissues by *E. coli* or *P. intermedia* LPS was augmented in STZ-diabetic mice, (iii) alongside the elevations of HDC activity, histamine levels increased in several tissues and in the blood, (iv) a significant augmentation of the LPS-induced HDC elevation could be observed as early as 2 days after the STZ injection, (v) such an augmentation was not detectable in nondiabetic (i.e., nonhyperglycemic) mice that had been given a low dose of STZ, and (vi) while NA inhibited both the development of STZ-induced hyperglycemia and the augmentation of the LPS-induced HDC elevation, NAC had neither of these suppressant effects. (vii) NA reduced the histamine increases induced by STZ alone and by STZ + LPS. In the following paragraphs, we discuss these results.

4.1. Enhancement of HDC activity and histamine production in STZ-diabetic mice

It has been shown that histamine concentrations are elevated in plasma and tissues in STZ-induced diabetic rats [44] as well as in patients with diabetes mellitus [45]. It has also been shown that histamine synthesis is increased in various cells or tissues in STZ-induced diabetic rats (such as endothelial cells and adjacent smooth muscle cells [46], kidney [47,48], retina [49] and other tissues (lung and heart, but not brain, stomach, or skin) [47]). In addition, an agent inhibiting HDC has been reported to reduce the aortic accumulation of albumin in STZ-diabetic rats [50–52]. Our results using the mouse are in agreement with these results.

4.2. Effects of NA on HDC activity in STZ-diabetic mice

Before discussing the mechanism by which the LPS-induced HDC elevation might be augmented in STZ-diabetic mice, it is necessary to outline the mechanisms underlying the STZ-induced diabetes and the protective effect of NA (Fig. 7).

STZ is taken up selectively by islet β-cells through the glucose transporter GLUT-2 [53]. It has been suggested that STZ may cause damage to DNA by alkylation via methyl radicals [43,54]. However, more recent studies suggest that STZ may also damage DNA through reactive oxygen species, including nitric oxide (NO) [55–58]. The STZ-induced DNA-strand breaks lead to the activation of poly (ADP-ribose) synthetase (PARS, a DNA-repairing enzyme) [59]. This consumes its substrate, nicotinamide adenine dinucleotide (NAD), resulting in a profound intracellular depletion of NAD and leading to the death of islet β-cells, resulting in diabetes [35,60,61]. However, it is also supposed that the reactive oxygen species produced by STZ may contribute to the damaging of β-cells through their own cytotoxicity (Fig. 7). In addition, it is likely that STZ is taken up, although possibly only to a small extent, by cells other than islet β-cells, and that this produces unidentified effects related to the complications of diabetes.

NA can prevent the decrease in NAD and suppress the diabetogenic activity of STZ [59,62]. Although NA can be enzymatically converted to NAD, the mechanism by which NA prevents diabetes has been shown to involve an inhibition of PARS [41–43] (Fig. 7). Actually, NA does not inhibit (in fact, it enhances) STZ-induced single-strand DNA breaks [41], and combined administration of NA + STZ for a long period frequently induces β-cell tumors [63]. On the other hand, NA has been shown to

![Fig. 6. Effects of STZ, LPS, and NA on histamine levels. Experimental conditions were the same as in the experiments shown in Figs. 2 and 5. Each value is mean ± S.D. from 5 to 10 mice. *P < 0.05, **P < 0.01 vs. S + S → S. #P < 0.05, ###P < 0.01 between the two groups indicated.](image)

**Fig. 6.** Effects of STZ, LPS, and NA on histamine levels. Experimental conditions were the same as in the experiments shown in Figs. 2 and 5. Each value is mean ± S.D. from 5 to 10 mice. *P < 0.05, **P < 0.01 vs. S + S → S. #P < 0.05, ###P < 0.01 between the two groups indicated.

**Fig. 7.** Accepted mechanism by which STZ induces diabetes. See text for explanation.
reduce alloxan-induced DNA breaks [41] through its ability to scavenge reactive oxygen species [64]. Thus, this scavenging effect of NA may also be involved in reducing the STZ-induced DNA breaks and cytotoxicity (Fig. 7).

In the present study, NA suppressed both the elevation of blood glucose and the augmentation of the LPS-induced HDC elevation seen in STZ-induced diabetic mice. This result does not, however, mean that hyperglycemia itself is responsible for the augmentation because, as described above, NA also scavenges reactive oxygen species. NAC is also a potent scavenger of reactive oxygen species [65]. However, there are no available data showing that NAC inhibits PARS. In the present study, prior administration of NAC was not effective at all at reducing STZ-induced hyperglycemia, thus supporting the notion that the major mechanism by which STZ induces hyperglycemia is DNA alkylation via methyl radicals, not the production of reactive oxygen species (a conclusion also reached by Uchigata et al. [43,54]). In addition, our results suggest that STZ-generated reactive oxygen species (even if produced) may be involved neither in the basal enhancement of HDC activity nor in the augmentation of the LPS-induced HDC elevation. Thus, the effects of STZ on HDC activity may be due to its hyperglycemic action per se or possibly to a sequence of events induced by the hyperglycemia (including a disturbance of homeostasis due to hyperglycemia). If this is in fact the case, then such an augmentation of the effect of LPS would be expected to occur in other types of diabetes, such as type 1 diabetes (IDDM) and type 2 diabetes (NIDDM). Interestingly, Soriano et al. [66] reported recently that PARS inhibitors can reverse the established endothelial dysfunction seen in STZ-diabetic mice. It would be very interesting to examine whether NA is effective at reducing basal HDC activity or any existing augmentation of LPS-induced HDC activity when given after the establishment of diabetes.

4.3. Cells in which HDC activity is enhanced in STZ-diabetic mice

We tried in some additional experiments to detect HDC by immunohistochemical staining and to detect HDC mRNA by in situ hybridization. However, we failed to visualize the cells that produce HDC possibly because the amounts per cell were small or for some other unknown reasons. Therefore, we can as yet only speculate as to the types of cells in which HDC is induced. As shown in Fig. 6, STZ + LPS produced a marked histamine elevation in the blood and in all three organs tested, and STZ itself produced significant histamine elevations in the liver and lung as did LPS itself. It should be noted that the basal level of histamine in the spleen is markedly higher than in the liver. Previously, we have shown that the histamine level in the liver is low in both normal and mast cell-deficient mice, while the levels in the lung and spleen are much higher (six to seven times) in normal mice than in mast cell-deficient mice [67]. These results indicate that the liver of the mouse is poor in mast cells, while the lung and spleen are rich in these cells. Thus, at least in the liver, it seems likely that mast cells are not the major cells in which HDC is induced. In addition, because there is no detectable elevation in HDC activity in the blood in response to LPS [29], the increased histamine in the blood is likely to be derived from the tissues in which HDC is induced.

As described in our previous paper, a line of evidence suggests that the major cells in which HDC is induced in mice in response to LPS or proinflammatory cytokines (IL-1 and/or TNF) are vascular endothelial cells in various tissues and hematopoietic precursor cells in hematopoietic organs (spleen and bone marrow) [29]. Indeed, LPS induces HDC activity in various organs even in mast cell-deficient W/Wv mice, and the HDC induction in W/Wv mice is rather higher than that seen in normal control mice [68].

The major reason why a histamine increase is undetectable in the spleen in mice given LPS or STZ alone may be that there is a large amount of histamine in mast cells and a wide variation among individuals. This may mask a relatively small increase in histamine produced by the induction of HDC.

4.4. Mechanism underlying the STZ-induced enhancement of HDC activity and the sensitization of mice to the HDC-inducing action of LPS

HDC can be induced by a variety of cytokines, such as IL-1, TNF, G-CSF, GM-CSF, IL-3, IL12, and IL-18 [69,70]. Interestingly, the production of TNF in response to LPS has been shown to be augmented in STZ-diabetic rats [37]. Our preliminary experiments showed that the LPS-induced production of IL-1 and TNF was enhanced in STZ-diabetic mice. However, the LPS-induced HDC induction was also augmented in mice that cannot produce either IL-1α or IL-1β. Although the detailed mechanism by which STZ may sensitize animals to a given HDC-inducing effect is not clear, an enhanced production of some of the cytokines mentioned above may be involved in the sensitization induced by STZ or hyperglycemia.

4.5. Mutual exacerbation between periodontitis and complications of diabetes

The biological activity of a given LPS depends on its structure [71], and we have shown that the ability of P. intermedia LPS to elevate HDC activity is weaker than that of E. coli LPS [25]. Interestingly, in the present study, it was shown that the elevation of HDC activity induced by P. intermedia LPS was also augmented in STZ-induced hyperglycemic mice. As emphasized by Herzberg and Meyer [72], it is likely that through daily oral hygiene procedures or dental surgical procedures, exposure to oral microorganisms, including P. intermedia, may occur repeatedly for a long period in patients with periodontitis. Recently, we showed that intragingival injection into mice of small doses
of *P. intermedia* LPS results in a marked induction of HDC in extraoral tissues (liver, lung, spleen, and mandible) [73]. Although the following idea is highly speculative at present, our findings, taken together with the reported actions of histamine and the relationship between diabetes and periodontitis, lead us to hypothesize that bacterial infections and the complications of diabetes may mutually exacerbate one another, at least in part because both lead to an induction of HDC. In this hypothesis, diabetes may result in an enhanced production of histamine via HDC-induction in various tissues. This may lead to a state involving suppressed Th1 responses (i.e., suppressed cellular immunity), which favors the proliferation of bacteria, such as the oral gram-negative bacteria involved in periodontitis. The consequent enhanced production of LPS may feed back to induce further HDC. This positive feedback cycle may be responsible for the augmentation of the LPS-induced HDC elevation seen in STZ-diabetic mice in the present study and, possibly, in part for the mutual exacerbation between periodontitis and the complications of diabetes. We think that this is the first hypothesis ever proposed to explain the mechanism underlying this mutual exacerbation.

### 5. Conclusion

Our present findings in STZ-induced diabetic mice suggest that either hyperglycemia itself or a hyperglycemia-initiated sequence of events may lead not only to an enhancement of basal HDC activity, but also to a sensitization of the animal to the HDC-inducing action of LPS.

### Acknowledgements

We are grateful to Dr Robert Timms for useful discussion and for editing the manuscript.

### References


[38] Y. Endo, Methods Enzymol. 94 (1983) 42–47.


