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Effects of sodium fusidate in animal models of insulin-dependent diabetes mellitus and septic shock

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SUMMARY

We have evaluated the effects of the novel immunosuppressant sodium fusidate (fusidin) in the non-obese diabetic (NOD) mouse and in D-galactosamine (D-Gal)-presensitized BALB/c mice challenged with the bacterial superantigen, *Staphylococcus aureus* enterotoxin B (SEB) or with the endotoxin, *Escherichia coli* lipopolysaccharide (LPS). The NOD mouse model has clinical and histoimmunological features similar to those of human insulin-dependent diabetes mellitus (IDDM). The SEB- and LPS-treated BALB/c mouse models exhibit pathogenic similarities with human septic shock conditions. In the NOD mouse, fusidin suppressed the spontaneous development of insulitis (mean inhibition 73%) and hyperglycaemia (IDDM incidence 25% versus 0%) when administered at 40 mg/kg five times weekly for 8 consecutive weeks from the fourth week of age; concurrently treated animals exhibited reduced percentages of splenic T lymphocytes. This anti-diabetogenic effect was confirmed in the accelerated model of diabetes induced in the NOD mouse with cyclophosphamide (CY) (IDDM incidence 55% versus 21–6% using dosages of fusidin from 40 to 80 mg/kg five times weekly); protection from IDDM development was achieved even when the drug (80 mg/kg/day) was first administered 7 days after CY challenge. In contrast, fusidin did not reverse hyperglycaemia when administered to CY-treated animals within 3 days of IDDM development. In the two models of septic shock, prophylactic treatment with fusidin, 80 mg/kg given three times for 2 days prior to D-Gal/SEB or D-Gal/LPS challenge, drastically reduced the lethality compared with D-Gal/buffer-treated mice. This effect may depend on the inhibitory action of fusidin on the secretion of cytokines such as interferon-γ and tumour necrosis factor-α, the serum levels of which were greatly diminished in the fusidin-treated mice (mean inhibition 50–90%). These results demonstrate that fusidin may have a role in the treatment of cell-mediated autoimmune diseases and cytokine-mediated infectious diseases in humans.

INTRODUCTION

Fusidin, the sodium salt of a tetracyclic triterpenoic acid, is an antibiotic used primarily for the treatment of *Staphylococcus* infections.[1,2] Recently, both fusidic acid and its sodium salt, sodium fusidate (fusidin), were found to exhibit immunosuppressive properties *in vitro*.[3] Fusidin diminishes the secretion of T- and B-cell stimulatory cytokines such as interleukin-1β (IL-1β), IL-2 and interferon-γ (IFN-γ), and also inhibits the thymocyte costimulatory activity of IL-1α, IL-1β and IL-6. In agreement with these experimental observations, preliminary clinical studies have shown that fusidin may favourably influence the course of immunoinflammatory disorders such as chronic endogenous uveitis, Behçet's colitis, Crohn's disease and insulin-dependent diabetes mellitus (IDDM).[4–7] Fusidin-treatment also protects diabetes-prone BioBreeding (DP-BB) rats from diabetes development when administered early in life and prior to disease development.[8] Considering the low toxicity of fusidin,[9] these data predict a role for this drug in the treatment of autoimmune diseases and perhaps other immuno-inflammatory diseases. We therefore studied the effects of fusidin in the non-obese diabetic (NOD) mouse and in two murine models of human septic shock conditions.

The NOD mouse is a well-known experimental model exhibiting histoimmunological and clinical similarities to IDDM in humans.[10] As in humans, IDDM appears to occur in these mice as a consequence of an autoimmune reaction against the pancreatic β-cells, mediated by mononuclear cells that selectively infiltrate the islets of Langerhans prior to and shortly after IDDM development (insulitis). Although these

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cells progressively accumulate in the islets by 4–5 weeks of age, a long prediabetic period occurs in NOD mice, and IDDM develops primarily in female NOD mice (70–80%) by 30–40 weeks of age. However, the disease may be provoked at an earlier age by administration of cyclophosphamide (CY), which is thought to inhibit suppressor cell functions.[11–13] CY thus induces an accelerated form of diabetes with immunopathogenic features similar to those provoking the spontaneous form of IDDM in these mice, with T cells and macrophages playing a major pathogenic role.[14,15] The capacity of these cells to secrete immunomodulating and inflammatory cytokines such as IL-1β, IL-6, tumour necrosis factor-α (TNF-α) and IFN-γ seems important,[16–21] and both forms of the disease may be prevented with immunosuppressive drugs such as FK506 and deoxyspergualin (DSP).[22–25] Consequently, CY-treated NOD mice provides a relevant *in vivo* model of human IDDM for the screening of immunosuppressive drugs that modulate cell-mediated and/or cytokine-mediated immunoinflammatory processes.

Conditions similar to septic shock in humans may be induced in mice by administration of *Staphylococcus aureus* enterotoxin B (SEB) or *Escherichia coli* lipopolysaccharide (LPS) to D-galactosamine (D-Gal)-sensitized BALB/c mice.[26] From the pathophysiological point of view, these conditions develop as a result of the release of cytokines from inflammatory cells activated by SEB or LPS. While these two experimental conditions are rapidly lethal for the animals (8–24 hr from challenge) and TNF is an essential mediator in both models, major pathogenic differences exist between them.[26,27] SEB appears to act as a superantigen interacting with T-cell receptor Vβ chains, causing massive release of T-cell derived cytokines such as IL-2, IFN-γ, TNF-α and TNF-β. The T-cell dependent nature of SEB-induced lethality is also consistent with the protective effect exhibited by cyclosporin A (CsA),[26,28] a selective suppressor of T cells,[29] and by the resistance of T-cell deficient severe combined immunodeficient (SCID) mice to the syndrome.[26] In contrast, LPS directly stimulates macrophages to release cytokines such as IL-1α, IL-1β, IL-6 and TNF, the latter being particularly important as a pathogenic factor in septic shock.[27] Furthermore, T cells do not appear to be important for the development of the LPS-induced shock syndrome, and LPS-induced lethality may be prevented with corticosteroids that inhibit macrophage cytokine production, but not with CsA; the syndrome may also be provoked in T-cell deficient SCID mice.[26]

In this paper, we demonstrate that fusidin, given under different experimental conditions, suppresses both biochemical, histological and clinical features of all three animal models of immunoinflammation.

MATERIALS AND METHODS

Drugs and reagents

Fusidin, in 10 mg/ml phosphate-buffered saline (PBS), was provided by Sigma-Tau (Pomezia, Rome, Italy) through Leo Pharmaceutical Products (Ballerup, Denmark). Dexamethasone (DEX; 40 mg/kg body weight, in PBS, given intraperitoneally) and CsA (60 mg/kg body weight, in olive oil, given intramuscularly), were purchased from Sigma (St Louis, MO) and Sandoz (Basel, Switzerland), respectively. CY, in 20 mg/ml sterile water, was provided by Schering-Plough (Milan, Italy). Fluorescein isothiocyanate (FITC)-conjugated rat monoclonal antibodies (mAb) recognizing mouse T lymphocytes (Thy-1.2), CD4 (L 3T4) and CD8 (Lyt-2) antigens, and the IL-2 receptor (CD25), were from Pharmingen (San Diego, CA). Goat anti-mouse immunoglobulin (Pharmingen) was used to determine the percentages of B lymphocytes. Kits for detection of mouse IFN-γ and TNF-α were from Holland Biotechnology (Leiden, the Netherlands) and Genzyme (Cambridge, MA), respectively, and used according to the manufacturers' recommendations. SEB, LPS (serotype 0127:B8) and D-Gal were from Sigma.

Mice

Female NOD mice and BALB/c mice were purchased from Bomholtgaard Breeding Centre (Ry, Denmark). The mice were maintained under standard laboratory conditions with food and water *ad libitum*, and allowed to adapt at least 1 week before starting the experiments.

IDDM models

Two groups of female NOD mice were given either fusidin, 40 mg/kg, or the same volume of PBS intramuscularly (i.m.) five times a week from 4–12 weeks of age (Table 1). The mice were examined for diabetes development once a week. Diabetes development was diagnosed on the basis of 2 consecutive days of glycosuria followed by fasting glycaemia above 11·8 mmol/l. At the end of the treatment period, the mice were killed by cervical dislocation. Pancreatic specimens and splenic lymphoid cells were collected and analysed to evaluate the degree of insulitis and the proportion of splenic lymphoid cell subsets.

Another set of experiments was performed to evaluate the capacity of fusidin to protect female NOD mice from the accelerated model of IDDM, which can be induced with CY. CY challenge was done in euglycaemic, 14–17-week-old mice by a single intraperitoneal (i.p.) injection of 300 mg/kg of CY at day 0. Several groups of animals were created, which were treated with PBS or fusidin (Table 2). At days + 14 and + 15, the mice were examined for diabetes development using the same criteria as described above. At this point, fusidin or PBS treatment was withdrawn, and all mice were killed for histological analysis of the pancreatic islets.

Immunofluorescence analysis

Splenic lymphoid cells were obtained as previously described[30] from 12-week-old female NOD mice treated for 8 consecutive weeks with fusidin or PBS. The proportion of mononuclear cell subsets was evaluated by direct immunofluorescence analysis. This was performed by incubating 10^6 splenic lymphoid cells with 10 μl FITC-conjugated anti-Thy1.2, anti-Lyt-2, anti-L 3T4, anti-CD25 and anti-immunoglobulin mAb that specifically recognize mouse T cells, CD8$^+$, CD4, CD25$^+$ cells and B cells, respectively. Cell fluorescence was evaluated by flow cytometry (FACS-Star; Becton-Dickinson, Mountain View, CA).

Histological examination of pancreatic islets

Histological examination of the pancreatic islets was performed in a blind fashion by two pathologists unaware of the status and/or the treatment of the animals, as described previously.[30] The degree of mononuclear cell infiltration was graded as follows: 0, no infiltrate; 1, periductular infiltrate; 2, peri-islet infiltrate; 3, intra-islet infiltrate; 4, intra-islet infiltrate associated

with β-cell destruction. The mean score for each pancreas was calculated by dividing the total score by the numbers of islets examined.

Septic shock models

A lethal syndrome with features similar to human septic shock was elicited in 6-week-old female BALB/c mice 8–24 hr after challenge with 20 mg D-Gal subcutaneously (s.c.), together with either 50 μg LPS i.p. or 50 μg SEB i.p. (Table 3). To evaluate the effect of fusidin prophylaxis, two groups of mice were treated as above and, in addition, with 80 mg/kg fusidin given three times at −48, −24 and −2 hr prior to challenge. Untreated BALB/c mice and mice treated with CsA, DEX or PBS were included.

Finally, we studied the effects of fusidin on the accumulation of IFN-γ and TNF-α that occurs shortly (90–120 min) after injection of SEB (IFN-γ and TNF-α) or LPS (TNF-α). Experiments were therefore carried out in which BALB/c mice, pretreated with fusidin or PBS under the same experimental conditions, were killed 120 min after D-Gal/SEB or D-Gal/LPS challenge for serum collection and measurement of IFN-γ and TNF-α (Fig. 2).

Statistical evaluations

Statistical analyses were performed by chi-square and ANOVA. P-values < 0·05 were considered significant.

RESULTS

Fusidin suppresses histological and clinical signs of IDDM in NOD mice

Fusidin was administered for 8 consecutive weeks to female NOD mice after the age of 4 weeks. This time period was chosen because the extent of insulitis is mild or absent in 4-week-old NOD mice, with severe insulitis occurring in most mice from 10–12 weeks of age.[10] As expected, all mice treated with PBS developed signs of severe insulitis by the end of the treatment period, often associated with complete destruction of the pancreatic β-cells (Table 1). Moreover during this period five out of 20 (25%) mice developed overt diabetes with glycosuria and hyperglycaemia. In contrast, although fusidin prophylaxis could not completely suppress insulitis development, it drastically reduced its severity, and none of the treated animals developed diabetes (Table 1). Along with the protective effect on insulitis development, prolonged treatment with fusidin also reduced the percentages of splenic T lymphocytes, including $CD4^+$ and $CD8^+$ cells. The percentage of B lymphocytes was unaffected by the drug, but fusidin tended to decrease the proportion of activated spleen lymphoid cells expressing the IL-2 receptor (Fig. 1).

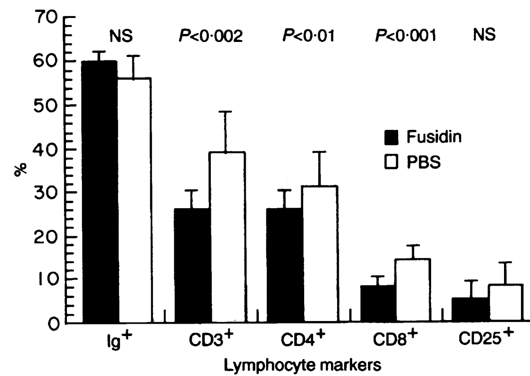

Figure 1. Splenic mononuclear cell subsets in NOD mice treated with fusidin or PBS. After 8 weeks of treatment with 40 mg/kg fusidin or PBS, eight euglygaemic female NOD mice in each experimental group were killed and spleen cells removed for immunofluorescence analysis. The fluorescence of cells was evaluated by flow cytometry. The results are expressed as mean values ± SD. Statistical analysis was performed by ANOVA; NS, not significant. Ig, immunoglobulin.

These observations prompted further assessment of the immunosuppressive properties of fusidin using the accelerated IDDM model induced in this strain of mice with CY.

When CY-challenged NOD mice were either untreated or only received PBS, an acute form of diabetes with severe insulitis, hyperglycaemia and glycosuria occurred 15 days after challenge in the majority of the animals (Table 2). Prophylactic treatment with fusidin given under different experimental conditions suppressed dose-dependently the development of clinical and histological signs of IDDM, thus substantiating the findings obtained in the spontaneous IDDM model. Protection from IDDM was also achieved when treatment with the highest

Table 1. Fusidin prophylaxis protects NOD mice from spontaneous development of diabetes

Treatment	Insulitis	*P* (ANOVA)	Diabetes incidence	*P* (chi-square)
PBS alone	2·6 ± 0·8	Control	5/20 (25%)	Control
Fusidin, 40 mg/kg	0·7 ± 0·3	<0·01	0/20 (0%)	<0·02

Fusidin prophylaxis was started in euglycaemic female NOD mice at 4 weeks of age. The drug was administered i.m. once daily, five times a week for 8 consecutive weeks. At the end of the treatment period or at diabetes onset, pancreatic specimens were collected for histological analysis.

Table 2. Fusidin protects NOD mice from accelerated development of diabetes induced by CY

Treatment	Insulitis	*P* (ANOVA)	Diabetes incidence	*P* (chi-square)
PBS alone, day −3–+14	2·8 ± 0·7	Control	11/20 (55%)	Control
Fusidin, 10 mg/kg, day −3–+14	3·1 ± 0·8	NS	10/16 (62·5%)	NS
Fusidin, 40 mg/kg, day −3–+14	2·1 ± 0·7	NS	4/19 (21%)	< 0·03
Fusidin, 60 mg/kg, day +1–14	0·7 ± 0·5	< 0·01	2/20 (10%)	< 0·002
Fusidin, 80 mg/kg, day +7–14	2·7 ± 1·1	NS	1/18 (5·6%)	< 0·001

Fusidin was administered daily, five times a week; see Table 1.
Day 0 = day of challenge with 300 mg/kg CY i.p.
NS, not significant.

Table 3. Fusidin prophylaxis protects D-Gal sensitized BALB/c mice from exotoxin- and endotoxin-induced lethality

	Treatment							
SEB	•	•	•	•				
LPS					•	•	•	•
PBS	•				•			
CsA		•				•		
DEX			•				•	
Fusidin				•				•
Lethality (%)	100	0	0	0	100	100	0	0

Mice, 20 in each group, were injected with D-Gal, 20 mg s.c., along with 50 μg SEB or 50 μg LPS i.p., CsA (60 mg/kg), DEX (40 mg/kg) or fusidin (80 mg/kg) was administered at −48, −24 and −2 hr prior to challenge.

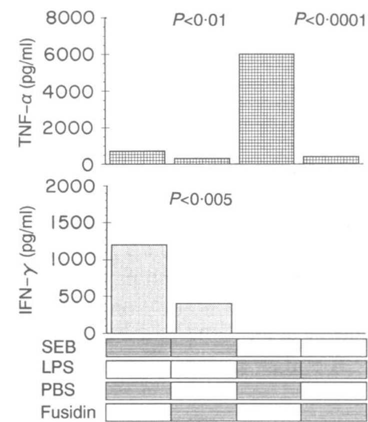

Figure 2. Fusidin suppresses the *in vivo* serum levels of IFN-γ and TNF-α. Two hours after challenge with D-Gal and either SEB or LPS (see Table 3), five mice from each experimental group were killed and individual serum samples were assayed for TNF-α and, in mice challenged with SEB, IFN-γ. Results are expressed as mean values. SD were < 15%. Statistical analysis was performed by ANOVA.

dose of fusidin was first started 7 days after CY challenge, even though this delayed treatment failed to reduce the signs of insulitis (Table 2). Fusidin, 80 mg/kg/day, failed to reverse hyperglycaemia when administered within 3 days of onset of overt diabetes (data not shown).

Fusidin side-effects in NOD mice

Prolonged treatment with fusidin for 8 consecutive weeks was generally well tolerated. None of the animals died during the treatment period, and histological analyses of their spleens, kidneys and livers did not show any pathological change compared to control mice. However, a modest but significant reduction in body weight was noticed in the mice treated with fusidin, 22·1 ± 3·1 g, compared with PBS-treated mice, 23·7 ± 2·1 g ($P < 0{\cdot}03$ by ANOVA). This also occurred in fusidin-treated mice challenged with CY (data not shown).

A common, dose-related side-effect occurring after injections of fusidin was an inflammatory reaction taking place at the site of injection, developing with variable severity in the animals. The i.m. route of administration was chosen because i.p. treatment with fusidin induces a granulomatous reaction, the s.c. route causes a severe inflammatory reaction, and fusidin is poorly adsorbed in mice after oral administration.[31] The possibility that the inflammatory reaction at the injection sites may have contributed to the immunosuppressive effects of fusidin seems unlikely. Thus, in each experimental group there was no correlation between the presence and/or severity of the reaction and clinical and/or histological effects of the drug.

Fusidin protects D-Gal-sensitized BALB/c mice from SEB- or LPS-induced lethality and suppresses the *in vivo* release of TNF-α and IFN-γ

Challenge with D-Gal/SEB or D-Gal/LPS caused the death of all control mice (untreated or PBS-treated) within 24 hr (Table 3). As previously reported by others,[26,28] pretreatment with DEX prevented the death of the mice in both experimental models, whereas CsA pretreatment only suppressed SEB, but not LPS, toxicity. In contrast, fusidin prophylaxis completely protected all animals from the lethal effect of both toxins (Table 3). Fusidin was, however, lacking its protective effects when treatment was first started 2 hr after endotoxin challenge (data not shown).

Since cytokines, particularly TNF and IFN-γ, are essential mediators of these syndromes,[26,27,32] we investigated whether fusidin inhibited the accumulation of these cytokines in the animals. As shown in Fig. 2, fusidin prophylaxis markedly diminished the serum levels of TNF-α and IFN-γ compared with PBS-treated controls.

DISCUSSION

Four classes of immunosuppressive drugs are commonly used for the treatment of autoimmune diseases and other immunoinflammatory disorders. These consist of corticosteroids, alkylating agents such as cyclophosphamide, anti-metabolites such as azathioprine and, used more recently, CsA. CsA is the prototype of a novel class of drugs, which include the macrolides FK506 and rapamycin. All these latter drugs seem to function through selective and reversible inhibition of T lymphocytes at non-toxic concentrations.[29]

In this study, we demonstrate that fusidin, like members of the above group of drugs, prevents both spontaneous and CY-induced diabetes in NOD mice, and also protects D-Gal-sensitized BALB/c mice from primarily T-cell (SEB-induced) and macrophage- (LPS-induced) dependent forms of lethality. The protective effect of fusidin on the development of histological and clinical signs of diabetes in the NOD mouse is in accordance with the anti-diabetogenic properties previously observed with this drug, both in newly diagnosed IDDM patients and in DP-BB rats.[7,8] In the lethality models, fusidin acted in a manner comparable to DEX, which prevented the death of mice in both the SEB and LPS models. In contrast, CsA only protected SEB-treated, but not LPS-treated, animals.

While the precise mechanism(s) of action of fusidin remains to be defined, *in vitro* studies of human mononuclear cells indicate that it does not exhibit a corticosteroid-like activity but

rather resembles CsA in its selective suppression of T-cell functions.[3] Our findings suggest that fusidin may function in a more complex manner *in vivo* than is predictable from *in vitro* experiments. For example, the drastic reduction of the serum levels of TNF-α observed in fusidin-treated mice after injections of SEB or LPS contrasts with the minimal efficacy with which the drug decreases TNF-α production *in vitro*.[3] Conversely, the capacity of fusidin to reduce the serum levels of IFN-γ in mice challenged with SEB agrees with previous *in vitro* experiments using mononuclear cells of human and rat origin.[3,8] Importantly, since both IFN-γ and TNF-α are involved in the development of IDDM and septic shock,[16,17,21,26,27,32] it is possible that this effect of fusidin may have contributed to the beneficial effects in these models. Moreover, fusidin reverses IL-1β- and IL-6-induced modulation of glucose-induced insulin production and inhibits IL-1β-induced IL-6 production by pancreatic islets of normal rats.[33] If the drug functions in a similar manner in mice, these effects may also have contributed to the protection of NOD mice from developing IDDM.

Fusidin-induced protection from IDDM may have involved two not mutually exclusive mechanisms, depending on whether the drug was given early or late during disease development. On the one hand, fusidin may have acted through a T-cell suppressive function which, in turn, inhibited/reversed the insulitis process (e.g. accumulation of mononuclear cells in the pancreatic islets) and the clinical appearance of hyperglycaemia. This mechanism fits with the outcome of the studies in CY-challenged and normal NOD mice receiving fusidin as a long-term prophylactic measure, since these animals showed milder signs of insulitis and, in the latter mice, had reduced numbers of splenic T cells. On the other hand, when administered in the efferent phase of the autoimmune reaction, fusidin may have prevented development of hyperglycaemia without interfering with the insulitis process. Hence, in the case of NOD mice first treated 7 days after CY challenge, fusidin-induced protection might have involved the antagonistic action of the drug on IL-1- and IL-6-induced dysfunction of the β-cells and/or suppression of effector cell functions.[33] This observation may be particularly relevant from the clinical point of view, where *prophylaxis* is feasible only in subjects exhibiting immunological and metabolic signs of ongoing insulitis. However, even though fusidin was incapable of reversing hyperglycaemia in diabetic mice with recent onset of CY-induced diabetes, the drug may also have a therapeutic role in human IDDM. In this regard, it is noteworthy that NOD mice as well as DP-BB rats with IDDM are less prone to remit than humans with recent onset IDDM. Also, the disease cannot be reversed in these animals by early treatment with CsA (F. Nicoleti *et al.*, manuscript submitted for publication), which often reverses human IDDM,[34–36] rapamycin,[37] FK506 and DSP (F. Nicoletti *et al.*, manuscript submitted for publication).

Positive effects of fusidin were achieved in a dose-dependent fashion in doses ranging between 40 and 80 mg/kg/day. However, caution should be exercised when extrapolating these results to the clinical setting, because fusidin is metabolized more rapidly in rodents than in humans, and lower doses of the drug are likely to exhibit immunosuppressive properties in humans.[9,31] This is underscored by preliminary studies of individuals with recent onset IDDM in which fusidin, at doses ranging between 20 and 40 mg/kg/day, favourably modulates the course of the disease (F. Nicoletti, unpublished observations).[7]

Fusidin has previously been shown to reduce the incidence of IDDM in DP-BB rats at lower doses (2–4 mg/rat/day, e.g. approximately 10–20 mg/kg body weight) than those presently used and, unlike in this study, fusidin was not preventing IDDM in a dose-dependent fashion.[8] Fusidin also induced anaphylactoid reactions in DP-BB rats when administered at doses higher than 4 mg/rat/day.[8] A probable explanation for these discrepancies is the different vehicles used for fusidin in the two studies, a mixture of olive oil (80%) and ethanol (20%) in the case of DP-BB rats[8] and PBS (this study). Thus, an anaphylactic reaction occurred in CY-challenged NOD mice treated with 1 mg/day of fusidin vehicled in olive oil/ethanol, and DP-BB rats treated with high doses of fusidin in PBS did not exhibit anaphylactoid reactions; in this case, the drug also displayed a dose-dependent effect on IDDM development (F. Nicoletti, unpublished observations).

It remains to be ascertained whether fusidin protects NOD mice from IDDM development in a temporary or permanent fashion. Although this issue was not addressed in this study, the reversibility of the anti-diabetogenic effect of fusidin both in newly diagnosed IDDM patients and in DP-BB rats after drug withdrawal (F. Nicoletti, unpublished observation)[8] makes it likely that diabetes will occur in NOD mice once fusidin treatment is interrupted. Recurrence of autoimmune diseases after withdrawal of CsA, FK506 and DSP is frequently observed both in humans and in experimental models.[8,30,37]

It is difficult to assess the therapeutic potential of fusidin in septic shock conditions. For example, DEX cannot reduce the mortality of septic shock, despite the efficacy by which it prevents endotoxin as well as exotoxin-induced lethality in mice.[38] This may be related to the mode of action of glucocorticoids on macrophages, particularly on the induction and secretion of TNF and IL-1, which are pivotal in the development of septic shock.[39] Thus, TNF- production can only be suppressed if steroids are given hours before the endo- or exotoxin challenge.[40] In this study fusidin behaved similarly, in that it failed to prevent both SEB- and LPS-induced lethality if administered 2 hr after challenge with either toxins. In contrast, fusidin administered up to two days before SEB or LPS completely prevented the lethal effects of the toxins and also abolished the *in vivo* production of TNF-α and IFN-γ. This observation suggests that in patients with specific shock, treatment with fusidin, like glucocorticoids, should be started immediately for some, if any, beneficial effects to be achieved. None the less, while these experimental studies do not anticipate a therapeutic role for fusidin in the management of septicaemia, this drug could still be considered for the prophylactic treatment of subjects at risk for developing both Gram-positive and Gram-negative forms of sepsis.

In conclusion, this study demonstrates that fusidin, a novel immunosuppressant of low toxicity, exhibits beneficial effects on experimental models of cell-mediated immunity and lethal immunoinflammation. Fusidin might therefore have an important role in the treatment of similar diseases in humans.

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REFERENCES

1. Godtfredsen W.O., Jahnsen S., Lorck H., Roholt K. & Tybring L. (1962) Fusidic acid: a new antibiotic. *Nature* **193,** 987.
2. von Daehne W., Godtfredsen W.O. & Rasmussen P.R. (1979) Structure–activity relationships in fusidic acid-type antibiotics. *Adv Appl Microbiol* **25,** 95.
3. Bendtzen K., Diamant M. & Faber V. (1990) Fusidic acid, an immunosuppressive drug with functions similar to cyclosporin A. *Cytokine* **2,** 423.
4. Bendtzen K., Vesti-Nielsen N., Petersen J., Andersen V. & Bendixen G. (1991) Treatment of chronic endogenous uveitis with fusidic acid. *Lancet* **337,** 552.
5. Langholz E., Brynskov J., Freund L.G. & Bendtzen K. (1991) Fusidic acid for Behcet's colitis: A novel approach to T-cell specific immunosuppressive therapy. *Dan Med Bull* **38,** 284.
6. Langholz E., Brynskov J., Bendtzen K., Vilien M. & Binder V. (1992) Treatment of Crohn's disease with fusidic acid: an antibiotic with imunosuppressive properties similar to cyclosporin. *Aliment Pharmacol Ther* **6,** 495.
7. Nicoletti F., Meroni P.L., Lunetta M. *et al.* (1991) Sodium fusidate and increased remission rate of insulin-dependent diabetes mellitus. *Lancet* **337,** 1292.
8. Nicoletti F., Di Marco R., Morrone S. *et al.* (1994) Reduction of spontaneous autoimmune diabetes in diabetes-prone BB rats with the novel immunosuppressant fusidic acid. Effect on T-cell proliferation and production of interferon-gamma. *Immunology* **81,** 317.
9. Reeves D.S. (1987) The pharmacokinetics of fusidic acid. *J Antimicrob Chemother* **20,** 467.
10. Lampeter E.F., Signore A., Gale E.A. & Pozzilli P. (1989) Lessons from the NOD mouse for the pathogenesis and immunotherapy of human type 1 (insulin-dependent) diabetes mellitus. *Diabetologia* **32,** 703.
11. Harada M. & Makino S. (1984) Promotion of spontaneous diabetes in non-obese diabetes-prone mice by cyclophosphamide. *Diabetologia* **27,** 604.
12. Yasunami R. & Bach J.F. (1988) Anti-suppressor effect of cyclophosphamide on the development of spontaneous diabetes in NOD mice. *Eur J Immunol* **18,** 481.
13. Charlton B., Bacelj A., Slattery R.M. & Mandel T.E. (1989) Cyclophosphamide-induced diabetes in NOD/WEHI mice. Evidence for suppression in spontaneous autoimmune diabetes mellitus. *Diabetes* **38,** 441.
14. Charlton B., Bacelj A. & Mandel T.E. (1988) Administration of silica particles or anti-Lyt2 antibody prevents β-cell destruction in NOD mice given cyclophosphamide. *Diabetes* **37,** 930.
15. Bacelj A., Charlton B. & Mandel T.E. (1989) Prevention of cyclophosphamide-induced diabetes by anti-V β8 T-lymphocyte-receptor monoclonal antibody therapy in NOD/Wehi mice. *Diabetes* **38,** 1492.
16. Bendtzen K. (1989) Immune hormones (cytokines); pathogenic role in autoimmune rheumatic diseases and endocrine diseases. *Autoimmunity* **2,** 177.
17. Mandrup-Poulsen T., Helqvist S., Mølvig J., Wogensen L.D. & Nerup J. (1989) Cytokines as immune effector molecules in autoimmune endocrine diseases with special reference to insulin-dependent diabetes mellitus. *Autoimmunity* **4,** 191.
18. Castaño L. & Eisenbarth G.S. (1990) Type-1 diabetes: a chronic autoimmune disease of human, mouse, and rat. *Annu Rev Immunol* **8,** 647.
19. Campbell I.L., Kay T.W.H., Oxbrow L. & Harrison L.C. (1991) Essential role for interferon-γ and interleukin-6 in autoimmune insulin-dependent diabetes in NOD/Wehi mice. *J Clin Invest* **87,** 739.
20. Debray-Sachs M., Carnaud C., Boitard C., Cohen H., Gresser I., Bedossa P. & Bach J.F. (1991) Prevention of diabetes in NOD mice treated with antibody to murine IFNγ. *J Autoimmun* **4,** 237.
21. Rabinovitch A. (1993) Roles of cytokines in IDDM pathogenesis and islet β-cell destruction. *Diabetes Rev* **1,** 215.
22. Miyagawa J., Yamamoto K., Hanafusa T. *et al.* (1990) Preventive effect of a new immunosuppressant FK-506 on insulitis and diabetes in non-obese diabetic mice. *Diabetologia* **33,** 503.
23. Carroll P.B., Strasser S. & Alejandro R. (1991) The effect of FK 506 on cyclophosphamide-induced diabetes in the NOD mouse model. *Transplant Proc* **23,** 3348.
24. Strandell E. & Sandler S. (1992) Protection against hyperglycemia in female nonobese diabetic mice treated with 15-deoxyspergualin. *Autoimmunity* **14,** 159.
25. Nicoletti F., Borghi M.O., Meroni P.L. *et al.* (1993) Prevention of cyclophosphamide-induced diabetes in the NOD/WEHI mouse with deoxyspergualin. *Clin Exp Immunol* **91,** 232.
26. Miethke T., Wahl C., Heeg K., Echtenacher B., Krammer P.H. & Wagner H. (1992) T cell-mediated lethal shock triggered in mice by the superantigen staphylococcal enterotoxin B: critical role of tumor necrosis factor. *J Exp Med* **175,** 91.
27. Tracey K.J., Fong Y., Hesse D.G. *et al.* (1987) Anti-cachectin/TNF monoclonal antibodies prevent septic shock during lethal bacteraemia. *Nature* **330,** 662.
28. Gonzalo J.A., Gonzalez-Garcia A., Kalland T., Hedlund G., Martinez C. & Kroemer G.. (1993) Linomide, a novel immunomodulator that prevents death in four models of septic shock. *Eur J Immunol* **23,** 2372.
29. Thomson A.W. (1992) The spectrum of action of new immunosuppressive drugs. *Clin Exp Immunol* **89,** 170.
30. Nicoletti F., Meroni P.L., Di Marco R. *et al.* (1992) The effects of deoxyspergualin on the development of diabetes in diabetes-prone BB rats. *Scand J Immunol* **36,** 415.
31. Findon G., Miller T.E. & Rowe L.C. (1991) Pharmacokinetics of fusidic acid in laboratory animals. *Lab Anim Sci* **41,** 462.
32. Heinzel F.P. (1990) The role of IFN-γ in the pathology of experimental endotoxemia. *J Immunol* **145,** 2920.
33. Bendtzen K., Diamant M., Horn T., Pedersen C. & Buschard K. (1992) Effect of fusidic acid on interleukin-1 (IL-1)- and IL-6-induced pancreatic β-cell functions in rats. *J Endocrinol* **132,** 345.
34. Assan R., Feutren G., Debray-Sachs M. *et al.* (1985) Metabolic and immunological effects of cyclosporin in recently diagnosed type 1 diabetes mellitus. *Lancet* **i,** 67.
35. Feutren G., Papoz L., Assan R. *et al.* (1986) Cyclosporin increases the rate and length of remissions in insulin-dependent diabetes of recent onset. Results of a multicentre double-blind trial. *Lancet* **ii,** 119.
36. Dupré J., Stiller C.R., Gent M. *et al.* (1988) Effects of immunosuppression with cyclosporine in insulin-dependent diabetes mellitus of recent onset: the Canadian open study at 44 months. *Transplant Proc* **20,** 184.
37. Baeder W.L., Sredy J., Sehgal S.N., Chang J.Y. & Adams L.M. (1992) Rapamycin prevents the onset of insulin-dependent diabetes mellitus (IDDM) in NOD mice. *Clin Exp Immunol* **89,** 174.
38. Sprung C., Caralis P.V., Marcial E.H. *et al.* 1984. The effects of high-dose corticosteroids in patients with septic shock. A prospective, controlled study. *N Engl J Med* **311,** 1137.
39. Natanson C., Hoffman W.D., Suffredini A.F., Eichaker P.Q. & Danner R.L. (1994) Selected treatment strategies for septic shock based on proposed mechanisms of pathogenesis. *Ann Intern Med* **120,** 771.
40. Han J., Thompson P. & Beutler B. (1990) Dexamethasone and petoxifylline inhibit endotoxin-induced cachectin/tumor necrosis factor synthesis at separate points in the signaling pathway. *J Exp Med* **172,** 391.