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Timing of pentoxifylline treatment determines its protective effect on diabetes development in the Bio Breeding rat

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Abstract

Diabetes-prone Bio Breeding (DP-BB) rats spontaneously develop diabetes between 60 and 120 days of age. Diabetes-resistant (DR)-BB rats can be induced to develop diabetes by poly(I:C) and anti-RT6. Here, we studied the effect of pentoxifylline, a potent anti-inflammatory agent, on diabetes development in both BB rat models of insulin-dependent diabetes mellitus and investigated whether these effects were related to differential modulation of tumour necrosis factor (TNF)-α and interleukin-10. When DP-BB rats received pentoxifylline from day 60 onwards, diabetes development was delayed and reduced. The other treatment protocols had no effect. In DR-BB rats, pentoxifylline treatment resulted only in a delay of diabetes development. In both BB rat models, in vivo pentoxifylline treatment potently suppressed TNF-α, but only moderately affected interleukin-10 production in vitro. These results show that timing of pentoxifylline treatment determines its protective effect on diabetes development in DP-BB rats. The observed pentoxifylline-induced increase of the interleukin-10/TNF-α ratio might be a mechanism for protection or delay of the diabetes development.

Keywords: Diabetes; Pentoxifylline; Interleukin-10; TNF-α; BB, rat

1. Introduction

Diabetes-prone (DP)-BB rats spontaneously develop diabetes between 60 and 120 days of age (Mordes et al., 2001). Diabetes-resistant (DR)-BB rats can be induced to develop diabetes by depleting them of their regulatory $RT6^+$ T cells and injection of low dose poly(I:C) (Greiner et al., 1987a,b). Likewise, protection of DP-BB rats against the onset of diabetes can be achieved by injecting them with these regulatory $RT6^+$ T cells (Burstein et al., 1989).

In the DP-BB rat model of diabetes, an early time interval has been identified in which diabetes can be prevented. For example, thymectomy of DP-BB rats around 30 days of age prevents insulin-dependent diabetes mellitus (Mordes et al., 2001), whereas thymectomy at day 60 has no effect (Mordes et al., 2001; Like et al., 1982). Moreover, injection with $RT6^+$ T cells from 30 to 60 days of age prevents diabetes in DP-BB rats, while injection from 60 to 100 days does not influence the disease (Burstein et al., 1989). These findings indicate that the period between 30 and 60 days of age is critical for the development of diabetes in DP-BB rats.

DP-BB rats develop diabetes in an age range of 60–120 days. This stage of disease development is characterised by severe insulitis (Mordes et al., 2001). The islets of Langerhans are infiltrated by macrophages, Natural Killer (NK) cells and cytotoxic T cells leading to destruction of the β-cells (Mordes et al., 2001). Cytokines, particularly the proinflammatory ones such as tumour necrosis factor-alpha (TNF-α) and interleukin-1, are cytotoxic for the β-cells and induce the inflammatory cascade leading to β-cell destruction (Rabinovitch, 1998). Several reports show that increased expression of proinflammatory cytokines such as interleukin-12, TNF-α, interleukin-1 and interferon-γ is associated with β-cell destructive insulitis, whereas non-destructive insulitis is more associated with increased expression of anti-inflammatory type 2 cytokines such as interleukin-10 and interleukin-4 and the type 3 cytokine transforming growth factor (TGF)-β (Rabinovitch, 1998; Rabinovitch et al., 1996; Kolb et al., 1996).

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Immunosuppressive drugs like corticosteroids, cyclosporin and phosphodiesterase inhibitors are used for the treatment of various autoimmune diseases like rheumatoid arthritis, inflammatory bowel disease and multiple sclerosis (Bach 1993). Moreover, these drugs are also capable of preventing the development of experimental autoimmune diseases such as experimental autoimmune encephalomyelitis (EAE) (MacPhee et al., 1989), streptococcal cell wall-induced arthritis (Sternberg et al., 1989) and bacteria-induced intestinal inflammation (Herfarth et al., 1998). Suppression of inflammatory cytokines such as TNF-α, interferon-γ and interleukin-12 is one of the main mechanisms for the protective effect of these drugs in autoimmunity (Bach, 1993).

The phosphodiesterase inhibitor pentoxifylline is a potent suppressor of TNF-α and other proinflammatory cytokines both in vivo and in vitro (Tilg et al., 1993; Neuner et al., 1994; Bernard et al., 1995; Staak et al., 1997; Voisin et al., 1998). On the other hand, pentoxifylline is able to enhance the production of the anti-inflammatory cytokine interleukin-10 in vitro (Platzer et al., 1995). Therefore, selective modulation of these cytokines by pentoxifylline might be one of the mechanisms in the protective effects of pentoxifylline in the induction of experimental autoimmune diseases like EAE (Rott et al., 1993).

In this study, we investigated the effect of pentoxifylline on diabetes development in the BB rat. In this study, we performed tests to determine in which phase of disease development treatment with pentoxifylline can prevent or delay the development of diabetes and whether this involves a selective regulation of TNF-α and interleukin-10.

2. Materials and methods

2.1. Animals

DP-BB and DR-BB rats were kept under viral antibody-free conditions in the Central Animal Facility of the University of Groningen and fed standard chow (Hope Farms, rodent diet no. Rmh-B2181, Woerden, the Netherlands) and acidified water ad libitum. In this study, rats of both sexes were used. In our colony, over 90% of DP-BB rats spontaneously developed diabetes before 130 days of age, regardless of gender.

Diabetes was induced in DR-BB rats by intraperitoneal (i.p.) injection three times a week with anti-RT6 (0.4 mg purified DS4.23/100 g rat) and 0.125 mg/100 g rat poly(I:C) (batch no.: 038H4146, Sigma, St. Louis, MO, USA). In this study, all rats became diabetic within 8 weeks of the start of the anti-RT6 and poly(I:C) treatment. The treatment started at 30 days of age.

Rats were weighed three times a week and screened for hyperglycemia using blood glucose test strips (Roche Diagnostics, Almere, The Netherlands). Rats were diagnosed diabetic on the basis of a plasma glucose concentration of >11 mM on two consecutive occasions. Diagnosis was confirmed by histological inspection of pancreatic tissue obtained at autopsy.

All animals received humane care in compliance with the principles of laboratory animal care (NIH publication no. 85-23, revised 1985) and the Dutch law on experimental animal care.

2.2. Experimental design

2.2.1. DR-BB rat studies

DR-BB rats were treated with 80 mg/kg body weight (BW) pentoxifylline (Sigma). Pentoxifylline was administered i.p. simultaneously with poly(I:C) and anti-RT6. This concentration of pentoxifylline has been demonstrated to protect Lewis rats against the induction of EAE (Rott et al., 1993).

At 95 days after the start of pentoxifylline treatment, all animals were sacrificed and blood or tissue samples were taken for further analysis. By that time, all DR-BB rats were diabetic. Rats that had become diabetic earlier during the treatment period, were kept normoglycemic with exogenous insulin (subcutaneous pellets, release 1.5–2 IU/24 h; Linshin Canada, Scarborough, Ontario, Canada) while the treatment was continued until day 95. To acquire blood and tissue samples, rats were anesthetized using halothane (Zeneca, Ridderkerk, The Netherlands) and killed after the procedure with euthanasate (Zeneca).

In order to investigate the effect of in vivo pentoxifylline treatment in healthy DR-BB rats on the induction of interleukin-10 and TNF-α in vitro, four healthy DR-BB rats received i.p. 80 mg/kg BW pentoxifylline dissolved in PBS, or only PBS for 7 days. At the end of the treatment period, blood samples were collected and used for cytokine analysis.

2.2.2. DP-BB rat studies

DP-BB rats received pentoxifylline mixed with regular food used at the animal facility (Hope Farms, Woerden, The Netherlands) at a concentration of 1 mg/g. Normal ad libitum food intake of the rats was, on average, approximately 20–30 g/day. This food intake resulted in an uptake of pentoxifylline of approximately 80 mg/kg BW. DP-BB rats received pentoxifylline from weaning until day 130 (continuous treatment), from weaning until day 60 (short treatment) and from day 60 to day 130 (effector-phase treatment). At the end of the experimental period (day 130), blood was collected from rats and used for cytokine analysis. All remaining rats at that time were not diabetic.

In order to investigate the effect of in vivo pentoxifylline treatment in healthy DP-BB rats on the induction of interleukin-10 and TNF-α in vitro, four healthy 50-day-old DP-BB rats received i.p. 80 mg/kg BW pentoxifylline dissolved

in PBS, or only PBS for 7 days. At the end of the treatment period, blood samples were collected and used for cytokine analysis.

2.3. Pancreas histology

Upon autopsy, the pancreas was removed and cleaned of fat and lymph nodes, fixed in Bouin's solution and processed for histological analysis. Sections (7 μm) were stained with hematoxyllin and eosin for evaluation of macrophage/mononuclear cell infiltration (insulitis) and degree of islet damage using a Zeiss Microscope. The degree of insulitis was rated on a scale of 1 to 4 as follows: 1, normal islet appearance and no infiltration; 2, mild insulitis where macrophages/mononuclear cells are around, but not in the islets; 3, severe insulitis, where macrophages/mononuclear cells completely penetrate and infiltrate the islets; 4, end stage islets. Per pancreas section an average histological insulitis score was calculated by adding up the histological insulitis score of each islet and dividing it by the total number of islets counted. Per section, a minimum of five islets were counted. Depending on the diabetes status of the animal, the number of islets per section ranged from 5 to 20. Analysis was performed independently by two persons.

2.4. Whole blood cultures

Heparinized whole blood was collected via cardiac puncture, five times diluted in RPMI (Gibco) supplemented with 60 μg/ml gentamycin, 2mM L-glutamine and 50 μM β-mercaptoethanol, and stimulated with 100, 500 and 1000 ng/ml lipopolysaccharide (*Escherichia coli*, serotype 0127:B8; Sigma) for 24 h to induce cytokine production. The number of leukocytes in the whole blood was determined using a Coulter counter (Coulter). After 24 h of culture, supernatant was collected and stored at -20° until cytokine assay.

2.5. Cytokine analysis

Interleukin-10 and TNF-α levels in the supernatants were measured by enzyme-linked immunosorbent assay (ELISA), using commercially available ELISA kits (OPTEIAI ELISA kits, Pharmingen, Becton Dickenson, The Netherlands) according to the manufacturer's instructions.

2.6. Statistical analysis

The product limit method of Kaplan and Meier was used to estimate diabetes incidence. Test groups were compared using the log rank test. The mean cytokine production between the groups was compared using the Mann–Whitney *U*-test. We considered *P* values of less than 0.05 to be significant. For statistical analysis, SPSS 8.0 software package for Windows was used.

3. Results

3.1. Pentoxifylline postpones the onset of diabetes in DR-BB rats

To investigate whether pentoxifylline can protect DR-BB rats from the induction of diabetes by anti-RT6 and low-dose poly(I:C), DR-BB rats were treated with 80 mg/kg BW pentoxifylline.

Fig. 1 shows that pentoxifylline significantly delayed the onset of diabetes development in the DR-BB rat ($P < 0.02$, log rank test, Kaplan Meier survival curves). Treatment with pentoxifylline increased the mean day of onset from 32 ± 3.3 days in the poly(I:C) + anti-RT6-treated group to 61 ± 12.8 days in the pentoxifylline + poly(I:C) + anti-RT6-treated group ($P < 0.05$, Mann–Whitney *U*-test). However, pentoxifylline did not prevent the induction of diabetes, because at 95 days after the start of the treatment all pentoxifylline-treated DR-BB rats had become diabetic.

3.2. Oral treatment of DP-BB rats with pentoxifylline reduces and delays diabetes development, but only when it is applied in the effector phase of disease development

We subsequently investigated the effect of pentoxifylline on the spontaneous development of diabetes in the DP-BB rat model. For this purpose, DP-BB rats received pentoxifylline mixed with their food in a concentration of 80 mg/kg BW as described in Materials and methods. The growth curves of rats that were given pentoxifylline were not different from the rats on regular chow (data not shown). The animals received pentoxifylline via their food in order to prevent effects of stress (caused by injections) on diabetes

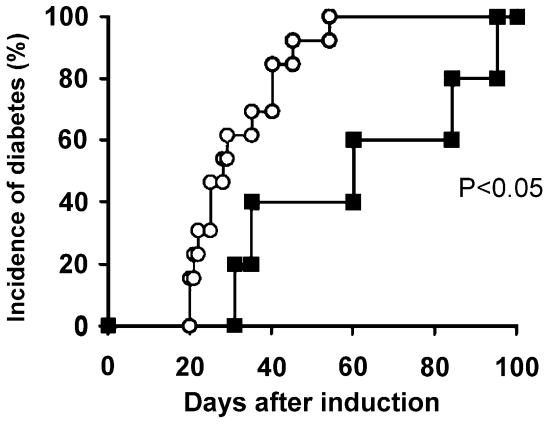

Fig. 1. Pentoxifylline postpones the onset of anti-RT6 and poly(I:C) induced diabetes in DR-BB rats. Diabetes was induced in DR-BB rats by i.p. injection of anti-RT6 (0.4 mg purified DS4.23/100 g rat and poly(I:C) (0.125 mg/100 g rat). Thirteen rats received anti-RT6 and poly(I:C) (○) only and five rats were treated with pentoxifylline (8 mg/100 g rat, injected simultaneously with anti-RT6 and poly(I:C) (■). Statistical significance was calculated using the log rank test (Kaplan Meier survival curves).

development in DP-BB rats. Fig. 2 shows that oral treatment of DP-BB rats with pentoxifylline significantly delayed and reduced diabetes development only when it was applied in the effector phase (from day 60 to day 130) of disease development ($P = 0.02$, log rank test, Kaplan Meier survival curves). This pentoxifylline treatment increased the mean day of onset from 81.7 ± 4.7 days in the control group to 97.8 ± 6.7 days in the effector phase-treated group ($P < 0.05$, Mann–Whitney U-test).

Treatment with pentoxifylline from weaning to day 60 or even throughout the experimental period (weaning to day 130) had no effect on the development of diabetes.

3.3. Non diabetic DP-BB rats treated with pentoxifylline in the effector phase of disease development have no insulitis, whereas the non-diabetic rats of the other groups show signs of insulitis

Among untreated DP-BB rats, all animals became diabetic before 130 days of age. Only 1 out 13 in the continuous treatment group did not develop diabetes, compared to 2 out of 14 in the short treatment group and 4 out of 12 in the effector-phase treatment group. At the moment of diagnosis of diabetes or at the end of the experimental period of 130 days, the pancreas was removed and processed for histological analysis.

As demonstrated in Table 1 no difference in the histological score of insulitis between the diabetic animals of various treatment groups was observed.

However, the non-diabetic animals in the effector-phase-treated group showed no histological signs of insulitis, whereas the non-diabetic animals of other groups showed histological signs of insulitis. These results suggest that the non-diabetic animals of other groups might have developed diabetes later, whereas the absence of insulitis in the non-diabetic rats of the effector-phase-treated group indicates complete protection against diabetes development.

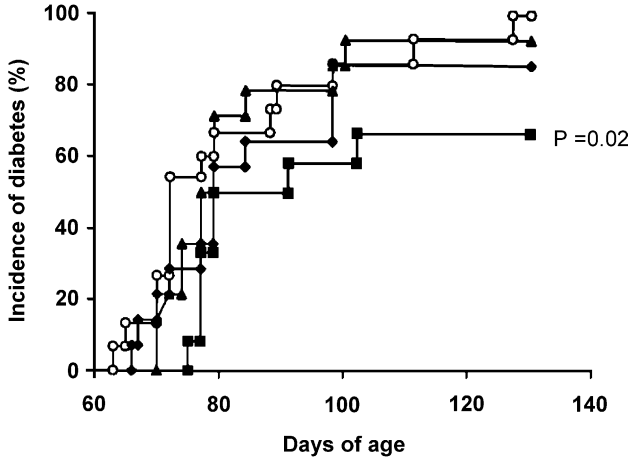

Fig. 2. Pentoxifylline treatment in the effector phase of disease development postpones and reduces diabetes development in the DP-BB rat. Pentoxifylline was administered (80 mg/kg BW) to DP-BB rats orally via their food from weaning until the end of the experimental protocol (continuous, $N = 13$, ▲), from weaning to day 60 (short, $N = 14$, ♦) or from day 60 to the end of the experimental period at day 130 (effector, $N = 12$, ■). The control group received the regular food used at the animal housing facilities (control, $N = 15$, ○). Statistical significance between the control group and the effector treatment group was calculated using the log rank test for Kaplan Meier survival curves.

Table 1
Histological insulitis score of the diabetic and non-diabetic rats in the different treatment groups

Treatment	(*n*)	Histological insulitis score			
		Diabetic	(*n*)	Non-diabetic	(*n*)
Control	15	2.68 ± 0.45	15		
Continuous	13	2.83 ± 0.37	12	1.86	1
Short	14	2.99 ± 0.40	12	1.50 ± 0.24	2
Effector phase	12	2.79 ± 0.77	8	1.07 ± 0.14	4

Data are expressed as mean ± S.D.

3.4. Differential effects of pentoxifylline treatment in vivo, on the induction of TNF-α and interleukin-10 in vitro

3.4.1. DR-BB rat studies

At 95 days after the induction of diabetes by anti-RT6 and Poly(I:C) treatment, all animals were sacrificed and blood samples were taken for further analysis. By that time, all DR-BB rats were diabetic. Rats that had become diabetic earlier during the treatment period were kept normoglycemic with exogenous insulin until day 95 while they still received anti-RT6 and Poly(I:C). Blood was obtained from all five pentoxifylline + poly(I:C) + anti-RT6-treated animals and of three poly(I:C) + anti-RT6-treated animals. Blood was placed in culture and stimulated for 24 h with lipopolysaccharide to establish TNF-α and interleukin-10 production. Three untreated 125-day-old DR-BB rats served as a control.

Fig. 3 shows that treating DR-BB rats with poly(I:C) and anti-RT6 is detrimental for interleukin-10 production in vitro. Interestingly, pentoxifylline treatment in vivo restored interleukin-10 production in vitro almost to the levels measured among untreated DR-BB rats. Treatment with poly(I:C) and anti-RT6 was also detrimental for TNF-α production, but pentoxifylline did not restore production to the levels as measured in untreated DR-BB rats. Fluorescence Activated Cell Sorter (FACS) analysis showed depletion of $RT6^+$ T cells from 49% to 19% and a relative increase of monocytes from 11% to 34% in the anti-RT6 + poly(I:C)-treated animals. Administration of pentoxifylline did not change this pattern (data not shown).

We subsequently investigated in normal DR-BB rats the effect of in vivo pentoxifylline treatment on the induction of interleukin-10 and TNF-α in vitro. In these experiments, BB rats received 80 mg/kg BW pentoxifylline dissolved in phosphate-buffered saline (PBS), or PBS only, in daily i.p. administration for 7 days. At the end of the treatment period, blood was collected and stimulated for 24 h with lipopolysaccharide to induce interleukin-10 and TNF-α production.

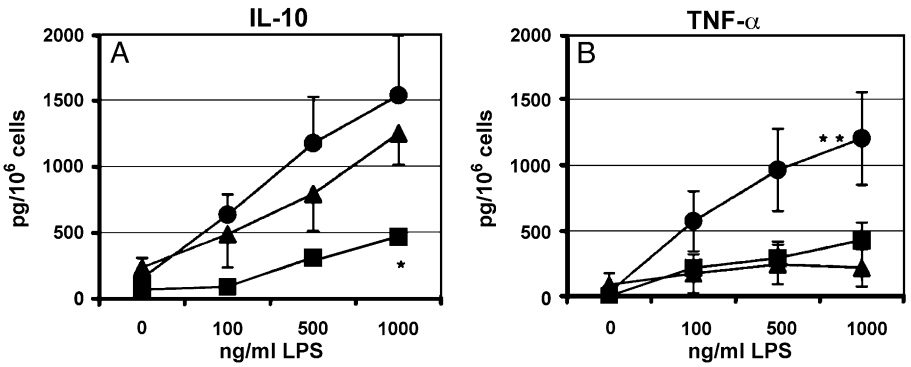

Fig. 3. Poly(I:C) and anti-RT6 treatment in vivo is detrimental for TNF-α and IL-10 production in vitro. Pentoxifylline treatment restores IL-10 production, but has no effect on TNF-α production in vitro. Heparinized whole blood was taken from five pentoxifylline + anti-RT6 + poly(I:C)-treated animals (▲) and from three anti-RT6 + poly(I:C)-treated animals (■). Three healthy untreated 125-day-old DR-BB rats served as controls (●). Whole blood was put in culture and stimulated with 100, 500 and 1000 ng/ml lipopolysaccharide to induce IL-10 (A) and TNF-α (B) production. Cytokines were measured in the supernatant by ELISA. Data are expressed as mean (± S.E.M.) pg/10^6 cells. * $P < 0.05$ as compared to (●) and (▲), (Mann–Whitney *U*-test). ** $P < 0.05$ as compared to (■) and (▲), (Mann–Whitney *U*-test).

As shown in Fig. 4A and B, in vivo treatment with pentoxifylline potently reduces TNF-α, but not interleukin-10 production, in vitro. Pentoxifylline treatment resulted in a two-fold reduction of the maximal inducible TNF-α production, whereas the production of interleukin-10 was not affected. In these animals, pentoxifylline did not affect the lipopolysaccharide-induced production levels of interleukin-10, but the spontaneous interleukin-10 production was slightly increased. Administration of pentoxifylline in vivo did not *result* in a change in the leukocyte number and

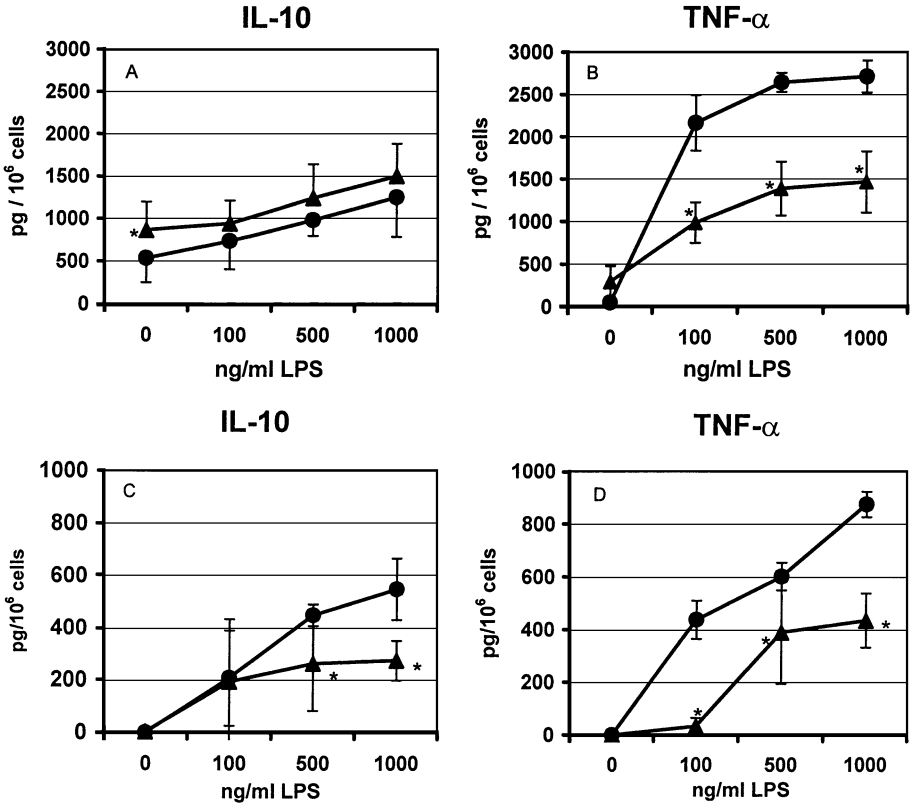

Fig. 4. Treatment with pentoxifylline in vivo is detrimental for TNF-α and beneficial for IL-10 production in vitro. Healthy DR-BB (A, B) and DP-BB (C, D) rats were injected i.p. with PBS ($N = 4$, ●) or 80 mg/kg body weight pentoxifylline dissolved in PBS ($N = 4$, ▲) for 7 days. After treatment, heparinized whole blood was obtained by cardiac puncture and stimulated for 24 h with 100, 500 or 1000 ng/ml lipopolysaccharide to induce IL-10 or TNF-α production. Cytokines were measured in the supernatant by ELISA. The data are expressed as mean (± S.E.M.) pg/10^6 cells. * $P < 0.05$ as compared to (●) (Mann–Whitney *U*-test).

the levels of monocytes, T cells, β-cells and CD4/CD8 ratio in whole blood as compared to PBS-treated animals (data not shown).

3.4.2. DP-BB rat studies

As in the DR-BB rat studies, we also investigated the effect of 7-day in vivo pentoxifylline treatment on the induction of interleukin-10 and TNF-α in vitro. In DP-BB rats (Fig. 4C,D), pentoxifylline treatment resulted in a reduced induction of both TNF-α and interleukin-10 in vitro. However, the effect on TNF-α was much more evident as compared to interleukin-10.

As can be seen in Fig. 4, diabetes-prone BB rats have a three-fold reduced cytokine production when compared to diabetes-resistant BB rats. This is probably due to the fact that diabetes-prone BB rats are naturally deficient of regulatory $RT6^+$ T cells, since the same phenomenon was also observed in diabetes-resistant BB rats after depletion of their $RT6^+$ T cells with anti RT6 (Fig. 3).

As in the DR-BB rat studies, administration of pentoxifylline in vivo to DP-BB rats did not result in a change in the leukocyte number and the levels of monocytes, T cells, β-cells and CD4/CD8 ratio in whole blood as compared to PBS-treated animals (data not shown).

4. Discussion

In this study, we have demonstrated that pentoxifylline reduces diabetes development in the BB rat, but only provides protection against spontaneous diabetes development when applied in the effector phase of disease development. Treatment of rats with pentoxifylline in vivo dramatically reduces the induction of TNF-α by lipopolysaccharide in vitro, but the induction of interleukin-10 remains mostly unaffected.

The effector phase of diabetes development after 60 days of age in the DP-BB rat is characterized by severe insulitis and destruction of the β-cells by cytotoxic T cells, macrophages and NK cells (Mordes et al., 2001). Furthermore, proinflammatory cytokines like TNF-α and interleukin-1, which are produced in high amount by these infiltrating cells, are cytotoxic for the β-cells (Rabinovitch, 1998; Saldeen, 2000). It is therefore likely that blocking these inflammatory responses will delay or prevent the total destruction of the β-cell and subsequently the onset of diabetes. Indeed, when we treat DP-BB rats in the effector phase of disease development with pentoxifylline, a potent suppressor of inflammation, the onset of diabetes is delayed and even prevented. However, continuous treatment of the DP BB rats with pentoxifylline or a short treatment during the insulin-dependent diabetes mellitus sensitive window did not influence the disease process. The absence of insulitis in the non-diabetic rats of the effector-phase-treated group indicates complete protection against diabetes development. Moreover, induction of diabetes in the DR-BB rat was also delayed by pentoxifylline.

In the induced model of diabetes development, the DR-BB rats are depleted of their regulatory $RT6^+$ T cells and poly(I:C) is added for activation of the immune system (Mordes et al., 2001; Greiner et al., 1987a,b; Sobel et al., 1992). Poly(I:C) is a synthetic double-stranded RNA, which mimics a viral infection. For instance, poly(I:C) has been demonstrated to be a potent inducer of TNF-α and interleukin-12 production and an activator of dendritic cells (Verdijk et al., 1999). Interestingly, we observed that treatment with poly(I:C) and αRT6 in vivo not only reduced the induction of interleukin-10, but also of TNF-α by lipopolysaccharide in vitro. Pentoxifylline was able to restore interleukin-10 production to the levels found in untreated healthy DR-BB rats, while TNF-α production remained low. This differential effect of pentoxifylline on interleukin-10 and TNF-α production was also demonstrated in whole blood cultures of DR-BB rats that were treated with pentoxifylline only. Because interleukin-10 can be protective and TNF-α detrimental in the effector phase of diabetes development (Rabinovitch, 1998), the enhancement of interleukin-10 and reduction of TNF-α will reduce the inflammatory cascade leading to the destruction of the β-cell. Therefore, the fact that pentoxifylline could only postpone diabetes development in DR-BB rats can be explained by the fact that pentoxifylline reduced the adjuvant effect of poly(I:C) but did not counteract the depletion of the regulatory $RT6^+$ T cells.

As shown in Fig. 4, suppression of TNF-α and the subsequent increase of the IL-10/TNF-α ratio might be the main reason why pentoxifylline prevents the induction of diabetes in the effector phase of disease development in the DP-BB rat. Accordingly, the protection of non-obese diabetic (NOD) mice against diabetes by pentoxifylline was related to the suppression of TNF-α and other proinflammatory cytokines (Liang et al., 1998). However, TNF-α exhibits paradoxical effects on diabetes development in both the NOD mouse and DP-BB rat model of diabetes. Accelerating or protective effects of TNF-α on diabetes development depends on dose and timing (Rabinovitch, 1998). For example, Satoh et al. (1990) demonstrated that administration of TNF-α from weaning onwards could prevent the onset of diabetes in the DP-BB rat. Intriguingly, the administration of other proinflammatory cytokines like interferon-γ also protects DP-BB rats from diabetes development (Nicoletti et al., 1998; Sobel and Newsome, 1997). Interestingly, when these proinflammatory cytokines are given in the effector phase of disease development, an acceleration of the disease can occur (Rabinovitch, 1998). For instance, in NOD mice, a very narrow time window is related to protection or acceleration of the disease by TNF-α (Yang et al., 1994; Green and Flavell, 2000; Green et al., 2000).

The anti-inflammatory cytokine interleukin-10, on the other hand, can provide protection against diabetes development (Pennline et al., 1994). Like TNF-α, interleukin-10 has

also been shown to exhibit paradoxical effects on the development of autoimmune diabetes (Rabinovitch, 1998). Timing and localisation of interleukin-10 distribution are of critical importance. For instance, local tissue expression of interleukin-10 in the pancreas accelerates diabetes development in NOD mice (Moritani et al., 1994), whereas systemic administration of interleukin-10 more in the effector phase of disease development rescues the NOD mice from diabetes development (Pennline et al., 1994).

Sobel et al. (2000) demonstrated that an induction of regulatory T cells by cyclophosphamide in the period between 30 and 60 days can prevent diabetes development in the DP-BB rat. Such an induction of a protective mechanism by TNF-α or interferon-γ when these cytokines are applied from weaning is proposed to be responsible for the prevention of diabetes in the DP-BB rat (Rabinovitch, 1998).

Interestingly, we observed in 130-day-old non-diabetic DP-BB rats much higher cytokine levels compared to non-diabetic 60-day-old DP-BB rats. As was described by Rabinovitch (1998) and Satoh et al. (1990), diabetes development in the DP-BB rat can be prevented by immunostimulation. Therefore, high cytokine levels in old non-diabetic DP-BB rats might represent a more active immune system and subsequently protect these DP-BB rats for diabetes development.

When pentoxifylline is applied from weaning, the production of TNF-α and interferon-γ is suppressed and subsequently, the induction of a protective mechanism. This suppression of a protective mechanism might be responsible for the paradoxical result that continuous treatment with pentoxifylline does not prevent diabetes in the DP-BB rat. Accordingly, it also suggests that prophylactic pentoxifylline treatment of children at risk for developing diabetes might not be beneficial as opposed to the observed preservation of β-cell function by pentoxifylline in newly diagnosed diabetes patients (MacDonald et al., 1994).

In conclusion, our results show that prevention of diabetes development by pentoxifylline depends on the timing of treatment. Obviously, the observed pentoxifylline-induced suppression of TNF-α and relative enhancement of interleukin-10 might be the underlying mechanisms for prevention or delay of diabetes.

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