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Letters

Serum interleukin-8 level is increased in diabetic patients

Dear Sir,

It is suggested, that polymorphonuclear neutrophils (PMN) have an important role in the pathogenesis of chronic complications of diabetes [1, 2]. PMN are cells of inflammatory response and once activated they show the ability to aggregate and adhere to the endothelium. Their stimulation results in reactive oxygen species (ROS) production, intensified arachidonic acid metabolism and secretion of enzymes from PMN's granules. Both ROS and PMN's enzymes could contribute to the destruction of endothelial cells [3]. PMN activation due to a stimulus has two faces. In certain situations these natural defence mechanisms could be harmful. In patients with diabetes circulating PMN activation can be observed on the one hand [4] and a weakened PMN response to stimuli on the other hand [2]. This is particularly evident in patients with poorly controlled diabetes who have a long history of the disease and chronic complications [1, 2]. Despite the great progress in understanding the pathogenesis of chronic complications over the last few years, many questions are still unanswered and the sequence of events is not known.

It seems that the observed PMN activation in diabetic patients is an important pathogenetic link of long-term complications of the disease.

Interleukin-8 (IL-8) is found to be one of the strongest factors activating PMN. Therefore, a study was carried out to evaluate the serum IL-8 concentration in patients with diabetes. The study was done with a group of 42 Type I diabetic patients (25 women and 17 men, aged 19–43 years, mean duration of disease 10.8 ± 8.2 years and HbA_{1c} 7.6 ± 0.8 %) and 20 Type II diabetic patients (12 women and 8 men, aged 42–63 years, mean duration of disease 9.8 ± 6.4 years and HbA_{1c} 7.8 ± 1.2 %). None of the patients showed evidence of an acute or chronic infection, renal failure or ketoacidosis. Additionally, 30 sex-matched healthy adults were used as control subjects. All patients were informed about the aim of the study and gave their consent. The study was approved by the regional ethics committee.

The serum concentration of IL-8 was measured with the use of the ELISA method utilizing commercial Endogen sets. Statistical analysis was done with the use of two-sided *p*-value *t*-test and Pearson's correlation test.

The serum concentration of IL-8 in healthy subjects was 39.93 ± 4.96 pg/ml. In patients with Type I and Type II diabetes the serum concentration of IL-8 was considerably higher [(160.29 ± 34.81 and 138.7 ± 32.8 pg/ml, respectively, $p < 0.05$, $p < 0.05$) Fig. 1]. Linear regression analysis shows a significant correlation between the IL-8 serum concentration and HbA_{1c} ($r = 0.52$, $p < 0.05$).

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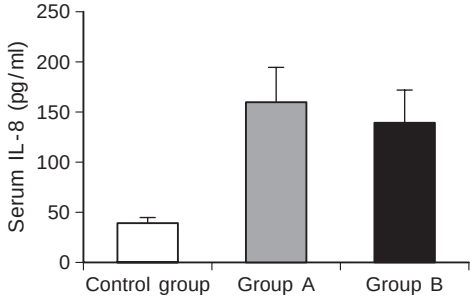

Fig. 1. Serum IL-8 concentration in healthy subjects (control group) and in Type I (group A) and Type II (group B) diabetic patients. Results are expressed as means ± SEM. Group A vs control group $p < 0.05$, group B vs control group $p < 0.05$, group A vs group B $p > 0.05$. □ control; ▨ Type I diabetes; ■ Type II diabetes

IL-8 is an important cytokine in the inflammatory process. Its main sources are macrophages, endothelial and epidermal cells. Initially it was suggested that IL-8 inhibits PMN adhesion and aggregation. Some authors noticed later, however, that IL-8 is a strong stimulator for PMN. Endothelial derived IL-8 secreted into subendothelial matrix or bound to the surface of the endothelium promotes neutrophils' adherence and migration. IL-8 is stimulated by high glucose concentrations in endothelial cells in vitro and has a chemotactic activity for PMN, lymphocyte T and smooth muscle cells [5–7] and a high expression of IL-8 in the atherosclerotic lesions was shown [6]. IL-8 could have an atherogenic role. Moreover, it was suggested that IL-8 participates in the pathogenesis of diabetic retinopathy because an increase of IL-8 concentration in the vitreous of patients with diabetic retinopathy was observed [7].

The results of this study indicate that the serum IL-8 concentration in Type I and Type II diabetic patients is markedly increased in comparison with healthy subjects and that it varies depending on the metabolic control of diabetes. The evaluation of this non-specific acute phase variable could be affected by subclinical infections. But in our study patients with either evidence of infection or latent sites of infection, such as urinary tract infection, chronic purulent tonsillitis or peridental abscessus, were excluded. These results might support other authors' opinions as described above about the important role of IL-8 in the development of late diabetic complications.

In our previous study, we showed that PMN are activated in diabetes [4]. The stimulation of circulating PMN and their impaired response to stimuli in diabetic patients depends on the metabolic control and the duration of diabetes [1, 2]. This study might elucidate the role of IL-8 in the stimulation of PMN.

In conclusion, the data show that in both types of diabetes mellitus the serum IL-8 concentration is elevated and that the inflammatory process and PMN probably play an important

role in the development of late vascular complications of diabetes.

Yours sincerely,
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Tetraploid cells and diabetic nephropathy

Dear Sir,
Polyploidy is an outstanding feature of vascular smooth muscle cells both in spontaneously hypertensive rats and in patients with essential hypertension [1, 2]. An increased number of tetraploid cells has been recently found in human skin fibroblast cultures from patients with Type I (insulin-dependent) diabetes mellitus and nephropathy, possibly in line with a similarity between cell abnormalities of diabetic nephropathy and those of essential hypertension [3, 4].

To understand whether this observation could be extended to fresh cells, we analysed the metaphases of fresh leucocytes and cultured human forearm skin fibroblasts in five patients with Type I diabetes and persistent proteinuria (3 men, 2 women, means ± SEM; median albumin excretion rate 1289.4 ± 262 μg/min; serum creatinine 117.9 ± 26 μmol/l) and in five age- and sex-matched patients with Type I diabetes but normoalbuminuria (median albumin excretion rate 5.06 ± 1 μg/min; serum creatinine 73.6 ± *8.8* μmol/l). Glycated haemoglobin concentrations were not raised in the patients with nephropathy (9.8 ± 0.5 vs 8.8 ± 0.3 %, p = NS).

Tetraploid cells could not be found in any sample of fresh leucocytes, although more than 100 cells were screened in each patient. Furthermore, tetraploid cells were present in similar number in the fibroblast cultures from the patients with nephropathy and from those with normoalbuminuria (1.2 ± 0.6 % vs 1.0 ± 0.5 %, means ± SEM, p = NS).

The overall number of tetraploid cells was lower in our patients than in two previously described and relatively larger groups of patients with similar characteristics (means ± SD: 25 ± 15 % vs 6 ± 4 %, $n = 10$ and $n = 7$, respectively), whose cells were cultured at higher glucose concentration [3].

Thus, to clarify whether an excess number of tetraploid cells could be ascribed to differences in glucose concentrations between culture media, we grew fibroblasts from two Type I diabetic patients with nephropathy and from two without renal complications in media with a high (20 mmol/l) or low (5 mmol/l) glucose concentration. No differences in the number of tetraploid cells were, however, detectable after 15 days, equivalent to two cultural passages (5 mol/l glucose: 2.3 ± 0.3 % vs 20 mmol/l glucose: 4.0 ± 0.6 % means ± SEM, $n = 4$, p = NS).

Proliferation rates, as measured over 7-day growth curves, were not related to the number of tetraploid fibroblasts in this small set of observations. The number of tetraploid cells was not associated with duration of fibroblast storage, nor with glycated haemoglobin concentrations.

Although sample size was relatively small, these results suggest that tetraploidy could be exceedingly rare in fresh cells from patients with Type I diabetes, and cultured skin fibroblasts are not more often tetraploid in diabetic nephropathy. Whether the excess number of tetraploid cells that was described previously could be explained by differences in experimental conditions that cannot be easily identified remains unclear, as glucose concentration in culture media was apparently the single technical condition at variance between this and previous studies [3, 4].

Yours sincerely,
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