Interleukin 1 beta (IL-1) and tumour necrosis factor alpha (TNF) are important for the beta cell lysis in insulin-dependent diabetes mellitus (IDDM), while IL-1 receptor antagonist (IL-1ra) is considered protective by blocking the effects of IL-1. Serum concentrations and ex-vivo production of IL-1, TNF and IL-1ra were examined in 10 newly diagnosed IDDM (ND-IDDM) patients, and compared with 11 long-standing IDDM (LS-IDDM) patients and 14 healthy volunteers. Ex-vivo LPS-stimulated production of IL-1 in ND-IDDM patients was significantly increased compared with LS-IDDM patients and healthy controls, while TNF and IL-1ra synthesis did not differ significantly. IL-1ra/IL-1 ratio was significantly decreased in ND-IDDM, and returned to normal values in the LS-IDDM group. Circulating concentrations of IL-1ra in LS-IDDM patients were increased. These data suggest a proinflammatory imbalance in ND-IDDM patients and this may play an important role in beta cell loss.

Insulin-dependent diabetes mellitus (IDDM) is an autoimmune disease in which beta cell lysis is the central event. In this process, the pro-inflammatory cytokines interleukin 1 beta (IL-1) and tumour necrosis factor alpha (TNF) are thought to play an important role. In vitro studies have shown that IL-1 interferes with insulin secretion from pancreatic beta cells and has a lytic effect on islet cells. Intraperitoneal administration of IL-1 accelerates the onset of IDDM in the BB-rat model, and induces hyperglycemia and hypoinsulinemia in normal Wistar Kyoto rats. Although TNF by itself is not cytotoxic to the beta cell, it strongly potentiates the action of IL-1. Interleukin 1 receptor antagonist (IL-1ra), the naturally occurring antagonist of IL-1, was shown to protect beta-cells against the deleterious effects of IL-1, and to delay the onset of diabetes in BB-rats.

The aim of the present study was to assess the pro- and anti-inflammatory cytokine production in IDDM patients. We investigated both circulating and ex-vivo produced IL-1β, TNFα and IL-1ra in newly-diagnosed IDDM (ND-IDDM) and long-standing IDDM (LS-IDDM) patients, and we compared them with a group of healthy volunteers.

RESULTS

Circulating concentrations of IL-1, TNF and IL-1ra

IL-1 concentrations were detectable in serum of only 2 of 10 healthy volunteers (4.2 and 5.6 pg/ml), 4 of 10 ND-IDDM patients (range 5–13.5 pg/ml) and 1 of 11 LS-IDDM patients (5.2 pg/ml). Circulating concentrations of TNF were below detection limit. Circulating IL-1ra concentrations did not differ between control individuals (234 ± 216 pg/ml) and ND-IDDM patients (184 ± 158 pg/ml), but were significantly higher in LS-IDDM patients (461 ± 167 pg/ml, P < 0.05) (Fig. 1A).
DISCUSSION

IDDM is characterized by mononuclear cell infiltration of the islets of Langerhans, associated with a selective β-cell lysis. Recent studies suggest that the pro-inflammatory cytokine IL-1 plays a major role in the inhibition of insulin secretion and in the lysis of β-cells. TNF, another major pro-inflammatory cytokine, strongly increases the effects of IL-1. In the present study we show that non-ketotic ND-IDDM patients have an increased production capacity of IL-1 and a tendency towards higher synthesis of TNF when compared with healthy controls. Apparently, the cytokine production capacity returns to normal values with longer duration of illness.

Contrasting data are available in the literature regarding the pro-inflammatory cytokine production capacity of IDDM patients. Some studies done in monocytes of ND-IDDM patients did not find any difference in IL-1 and TNF production when compared with the controls. However, the longer duration of diabetes (68.6 days) in the ND-IDDM patients investigated by Molvig and colleagues, and the use of silica as a stimulus for cytokine production by Luger et al., makes difficult the comparison of these studies with the present data. In contrast, other investigators have found an increased production of cytokines after in vitro stimulation of blood mononuclear cells of ND-IDDM patients as we also observed in our system. However, when comparing the present results with the data from the literature, it is also important to mention that the cytokine production capacity was assessed after whole blood stimulation, in the presence of all the cells and plasma factors normally present in circulation, which represents a system closer to the physiological conditions than the artificially isolated monocytes used in the above-mentioned studies. In this respect, the authors are aware that pancreatic cytokine determinations would have been more meaningful, but unfortunately there are no available methods to obtain pancreatic material from patients at the present moment.

Although metabolic disturbances cannot be ruled out as a factor in the observed differences in cytokine patterns between the ND-IDDM and the LS-IDDM patients, they are less probable to play a major role since the LS-IDDM patients studied had similar fasting blood glucose and glycated haemoglobin with the ND-IDDM group, and all IDDM patients were non-ketotic at the time of sampling.

In view of the reports that have shown that IDDM patients have an increased ex-vivo production of IL-1 beta and TNF alpha when compared with genotype-matched healthy controls, and the finding that TNF production is higher in individuals with DR3 and DR4 haplotypes, which are more prevalent in IDDM,
TABLE 1. Clinical characteristics of IDDM patients and healthy volunteers*

<table>
<thead>
<tr>
<th></th>
<th>ND-IDD M</th>
<th>LS-IDD M</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>10</td>
<td>11</td>
<td>14</td>
</tr>
<tr>
<td>Sex (M/F)</td>
<td>6/4</td>
<td>6/5</td>
<td>8/6</td>
</tr>
<tr>
<td>Age (years)</td>
<td>21 (13-23)</td>
<td>27 (12-35)</td>
<td>22 (19-27)</td>
</tr>
<tr>
<td>IDDM duration</td>
<td>3.4 days</td>
<td>4.7 years</td>
<td>—</td>
</tr>
<tr>
<td>Insulin (IU/day)</td>
<td>27.4 ± 12.9</td>
<td>49.9 ± 17.4</td>
<td>—</td>
</tr>
<tr>
<td>Fasting blood glucose (mg/dl)</td>
<td>260 ± 70</td>
<td>242 ± 89</td>
<td>94 ± 16</td>
</tr>
<tr>
<td>HBA1 (%)</td>
<td>9.4 ± 1.6</td>
<td>8.6 ± 1.1</td>
<td>7.1 ± 0.3</td>
</tr>
</tbody>
</table>

*Data are given as mean ± SD, with the exception of age which is given as median (interval).

Materials and Methods

Ten ND-IDD M patients, 11 LS-IDD M patients and 14 healthy individuals were studied. The clinical characteristics of the patients are given in Table 1. Written consent was given by each individual participating in the study. Both groups of IDDM patients were non-ketotic at the time of sampling. The C-peptide plasma concentration was tested in the IDDM patients and in all of them was below 0.1 nmol/l. Any sign of infection and/or other inflammatory diseases served as an exclusion criteria. The newly diagnosed IDDM patients were tested within 5 days from diagnosis, while each of the long-standing IDDM patients had more than 1 year duration of the disease.

Blood has been drawn from the cubital vein in 4-ml sterile tubes containing 48 µl EDTA (Vacutainer Systems, Becton and Dickinson, Rutherford, NJ). Plasma was separated and stored at —20°C until the assays were performed. All samples were collected between 7 and 8 a.m. The ex vivo lipopolysaccharide-stimulated cytokine production was assayed as described elsewhere.22 Briefly, the blood was drawn in another two tubes of 4 ml as described above. The tubes were incubated 24 h at 37°C with the addition of 50 µl of lipopolysaccharide (LPS) (LPS: E. coli serotype 055:B5, Sigma Chemical Co., St Louis, MO) containing 0.8 mg/ml (final concentration of LPS: 10 µg/ml). After 24 h, the tubes were centrifuged, plasma was collected and stored at —20°C until assay.

Measurement of IL-1β, IL-1α and TNF alpha. IL-1β and IL-1α concentrations were measured as duplicates using a quantitative enzyme immunoassay (Quantikine™, R&D Systems Europe, Abingdon, UK) according to the instructions of the manufacturer. Both kits used a specific monoclonal antibody bound to a solid phase, and a specific polyclonal antibody conjugated to horseradish peroxidase. The specified detection limit for IL-1β was 3.9 pg/ml, and for IL-1α was 46.9 pg/ml. TNF alpha was determined by an ELISA kit (Boehringer Mannheim, Germany), using two mouse monoclonal antibodies directed against two different epitopes of the human TNFα. The specified detection limit was 32 pg/ml. Samples from 5 ND-IDD M, 4 LS-IDD M and 10 healthy controls were also tested using a radio-immunoassay method, as described previously.7
**Statistical analysis**

Cytokine concentrations are expressed as mean ± SD. Differences between the groups were analysed using the Kruskal Wallis test with chi-square approximation. Differences were considered significant when P value was below 0.05.

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**REFERENCES**


