INTERLEUKIN 1β, TUMOUR NECROSIS FACTOR-α AND INTERLEUKIN 1 RECEPTOR ANTAGONIST IN NEWLY DIAGNOSED INSULIN-DEPENDENT DIABETES MELLITUS: COMPARISON TO LONG-STANDING DIABETES AND HEALTHY INDIVIDUALS

Mihai G. Netea,[1,3]   Nicolae Hancu,[1]   Willem L. Blok,[3]
Paula Grigorescu-Sido,[2]   Lenuta Popa,[2]   Victor Popa,[2]
Jos W. M. van der Meer[3]

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.[1] 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[2] and has a lytic effect on islet cells.[3] Intraperitoneal administration of IL-1 accelerates the onset of IDDM in the BB-rat model,[4] and induces hyperglycemia and hypoinsulinemia in normal Wistar Kyoto rats.[5] Although TNF by itself is not cytotoxic to the beta cell, it strongly potentiates the action of IL-1.[6] 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,[7] and to delay the onset of diabetes in BB-rats.[8]

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-ra 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).
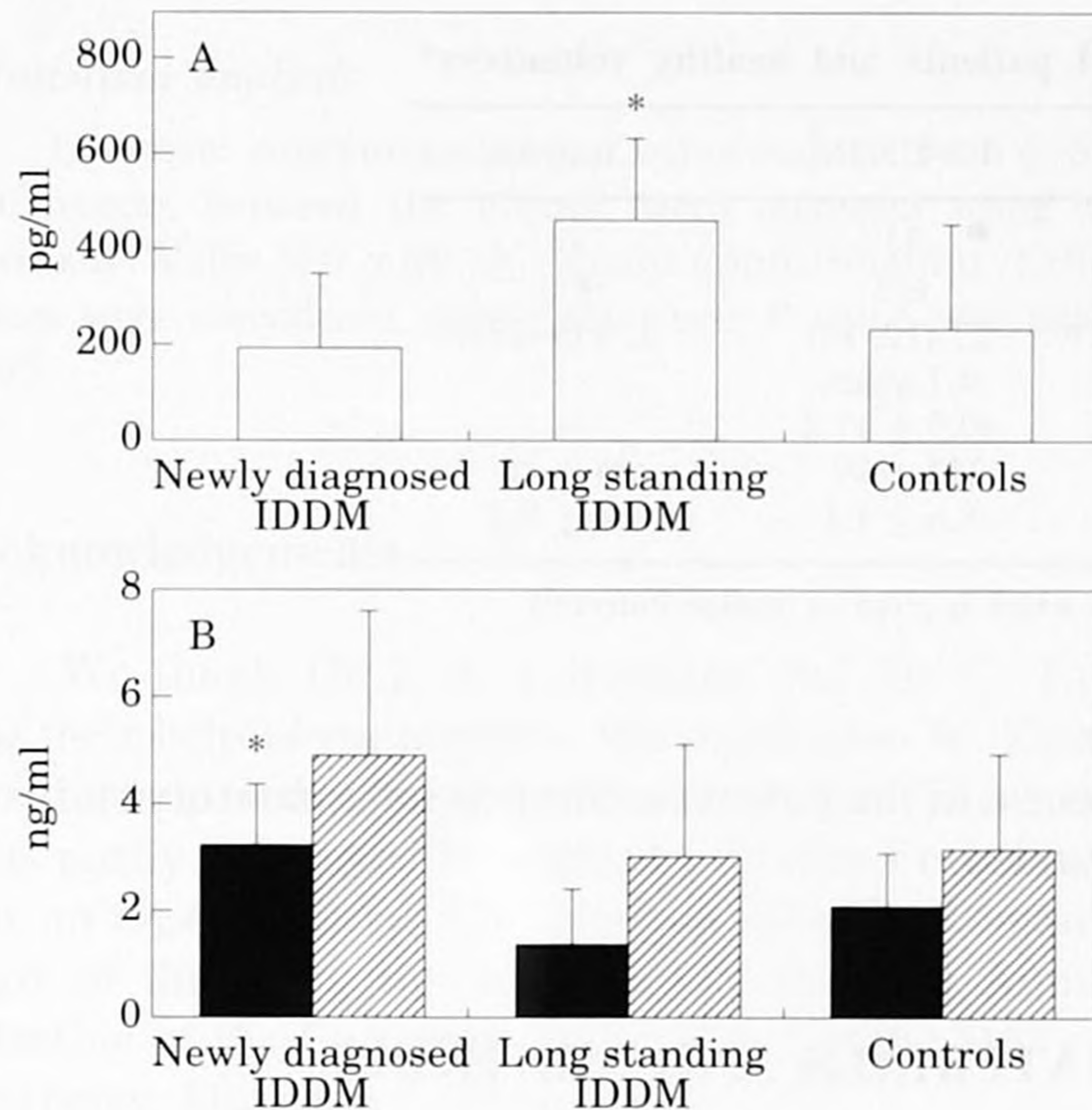
Figure 1. Circulating concentrations of interleukin 1 receptor antagonist (IL-1ra) (□) in healthy controls, newly diagnosed IDDM patients and long-standing IDDM patients (A) and ex-vivo production of interleukin-1 beta (IL-1) (■) and tumour necrosis factor alpha (TNF) (▨) after stimulation with LPS (10 μg/ml) (B).

Results expressed as mean ± standard deviation. *$P < 0.05$.

Ex vivo LPS-stimulated production of IL-1 in healthy volunteers was 2.1 ± 1.0 ng/ml. In ND-IDDM patients the release of IL-1 was significantly increased (3.2 ± 1.2 ng/ml, $P < 0.05$), whereas IL-1 production in LS-IDDM patients did not differ significantly from the control group (1.4 ± 1.0 ng/ml, $P > 0.05$) (Fig. 1B). Stimulated production of TNF was 3.1 ± 1.8 ng/ml in the control group; in ND-IDDM patients, TNF synthesis tended to be higher, but statistical significance was not achieved due to a wide range in the observations: 4.9 ± 2.7 ng/ml, $P > 0.05$. In LS-IDDM patients, TNF synthesis did not differ from controls: 3.0 ± 2.1 ng/ml, $P > 0.05$ (Fig. 1B). Stimulated pro-duction of IL-1ra did not differ between the three groups studied: 36.7 ± 9.3 ng/ml in healthy volunteers, 33.2 ± 9.9 ng/ml in ND-IDDM patients and 33.7 ± 14.3 ng/ml in LS-IDDM patients. Because an imbalance between the pro-inflammatory IL-1 and the anti-inflammatory IL-1ra may be involved in the pathogenesis of IDDM, the authors calculated the IL-1ra/IL-1 ratios: in healthy controls the ratio was 21.3 ± 8.9, in ND-IDDM it was strongly reduced: 11.5 ± 4.2 ($P < 0.02$), while in LS-IDDM patients the ratio did not differ significantly from controls: 32.1 ± 21.6 ($P > 0.05$).

Similar results have been obtained when the radio-immunoassay was used to determine the cytokine concentrations (data not shown), arguing for the accuracy of the commercially ELISA kits used for the determinations.

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[2] and in the lysis of β-cells.[3] TNF, another major pro-inflammatory cytokine, strongly increases the effects of IL-1.[6] 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.[9,10] However, the longer duration of diabetes (68.6 days) in the ND-IDDM patients investigated by Molvig and colleagues,[9] and the use of silica as a stimulus for cytokine production by Luger et al.[10] 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[11,12,13] 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,[14] 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*

	ND-IDDM	LS-IDDM	Controls
Number	10	11	14
Sex (M/F)	6/4	6/5	8/6
Age (years)	21 (13–23)	27 (12–35)	22 (19–27)
IDDM duration	3.4 days	4.7 years	—
Insulin (IU/day)	27.4 ± 12.9	49.9 ± 17.4	—
Fasting blood glucose (mg/dl)	260 ± 70	242 ± 89	94 ± 16
HBA$_1$ (%)	9.4 ± 1.6	8.6 ± 1.1	7.1 ± 0.3

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

it is possible that the "normal" production capacity with longer duration of illness represents a decrease of genetically increased production capacity. This hypothesis is sustained by other studies showing a decreased cytokine production capacity in patients with LS-IDDM.[15,16] Alternatively, the increased capacity found can be due to the disease process itself rather than to genetics.

To the best of the authors' knowledge, this study is the first to assess the ex vivo IL-1ra production capacity of IDDM patients. The stimulated production of IL-1ra did not differ between healthy individuals and ND- or LS-IDDM patients. The decrease of IL-1ra/IL-1 ratio in ND-IDDM patients sustains the hypothesis of Mandrup-Poulsen et al. that the imbalance between IL-1 and IL-1ra is central in the autoaggressive reaction against beta cell.[17] A similar imbalance of IL-1ra/IL-1 ratio was recently found in patients with chronic inflammatory bowel diseases,[18] in rheumatoid synovium[19] and in the synovial fluid of Lyme arthritis patients.[20]

Circulating concentrations of TNF were below the detection limit, as was the case for circulating IL-1 in the majority of samples. The differences in serum concentrations of IL-1ra are difficult to explain. While in newly diagnosed IDDM patients IL-1ra concentrations were similar to healthy individuals, we found significantly increased concentrations in long-standing IDDM. This is supported by the finding of higher IL-1ra circulating concentrations in IDDM patients with the A1/A2 genotype for IL-1ra gene when compared with control subjects with the same genotype.[21] Thus, it seems that differences in circulating IL-1ra concentrations do appear in the course of IDDM, and a correlation of these modifications with certain genotypes can not be excluded.

In conclusion, we have found an increased IL-1 production capacity and a decreased IL-1ra/IL-1 ratio in newly diagnosed IDDM patients, which argues for an imbalance in favour of the pro-inflammatory cytokines. This may be important in the pathophysiology of the beta cell lysis. During the evolution of the disease, probably after all the β-cells are lysed with a cessation of the inflammatory

process in the pancreas, this pro-inflammatory status is reversed.

MATERIALS AND METHODS

Ten ND-IDDM patients, 11 LS-IDDM 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 assesed 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-1ra and TNF alpha. IL-1β and IL-1ra 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-1ra 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-IDDM, 4 LS-IDDM and 10 healthy controls were also tested using a radio-immunoassay method, as described previously.[9]

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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