SHORT COMMUNICATION

Spontaneous production of interleukin 6 by adult T-cell leukaemia cells

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Interleukin-6 (IL-6) is a cytokine that has a wide variety of biological activities involved in the immune response, acute inflammation and haematopoiesis (Kishimoto, 1989). The cell types producing IL-6 are systemically distributed: T-cells, B-cells, monocytes, fibroblasts, keratinocytes, endothelial cells, and several tumour cells (Ray et al., 1989). IL-6 production in T-cells is induced by T-cell mitogens such as phorbolesters or concanavalin A and antigenic stimulation on direct contact with macrophages (Hori et al., 1988). Several T-cell lines, however, transformed by human T-cell lymphotropic virus type I (HTLV-I) express IL-6 mRNA without stimulation (Hirano et al., 1986; Noma et al., 1989). Adult T-cell leukaemia (ATL) is causally associated with HTLV-I infection and some ATL-cells produce or respond to lymphokines such as IL-1 (Kodaka et al., 1989), IL-2 (Tsuda & Takatsuki, 1983; Anma et al., 1987), and IL-4 (Uchiyama et al., 1988).

In this study, we attempted to elucidate whether ATL-cells secrete IL-6 or proliferate in response to this factor.

Blood samples were obtained from healthy volunteers and patients with ATL admitted to Kumamoto University Hospital between April 1988 and November 1989. The sera were cryopreserved at −80°C until IL-6 measurement. The mononuclear cells were separated from heparinised peripheral blood of six acute ATL (designated as ATL 1–6) and four normal controls and a cervical lymph node of one lymphoma type ATL (ATL 7) by gradient centrifugation on Ficoll-Hypaque. Surface phenotypes of the mononuclear cells as analysed by flow cytometry are shown in Table I. Furthermore, T-cell enriched preparations were obtained by a sheep red blood cell rosetting technique (Tsuda & Takatsuki, 1984). Purity of T-cells as evaluated by flow cytometry of FITC-conjugated anti-CD2-stained cells was 99% for ATL-cells and 90% for normal controls. T-cells were cultured in 96-well culture plates (200 µl per well) at a concentration of 1 x 10⁶ cells ml⁻¹ in RPMI 1640 containing 10% fetal calf serum in the absence of additional factors. Recombinant human IL-6 (1–2 x 10⁻¹⁵ m⁻²g) and polyclonal anti-human IL-6 antibody were obtained from Amersham (Arlington Heights, IL, USA) and R & D Systems (Minneapolis, MN, USA), respectively. An ELISA kit (Inter-Test 6, Genzyme Corporation, Boston, MA, USA) was used for the measurement of IL-6 in sera and conditioned media (CMs).

First, the sera of both healthy volunteers (n = 6) and ATL patients of different types or phases of disease (Kawano et al., 1985); acute (n = 9), chronic (n = 10), smouldering (n = 7), and lymphoma (n = 1) type, were tested for IL-6 levels. In the case of ATL, the patients with sepsis or endotoxaemia were omitted because serum IL-6 levels are known to be enhanced in such conditions (Hack et al., 1989). Results showed that not only control sera but also ATL sera from all four categories of ATL patient did not have detectable levels of IL-6 (data not shown) (limit of sensitivity is 0.163 ng ml⁻¹ or 0.815 u ml⁻¹).

Next, in order to clarify whether ATL-cells do secrete IL-6 in vitro, CMs sampled at 2, 4, 8, 24 and 24 h following culture were tested for IL-6 concentrations. As shown in Figure 1, two cases of ATL-CMs (ATL 1 and 2) showed obvious high levels of IL-6 as compared with CMs of normal T-cells at 8 and 24 h, and the case secreting the largest amount of IL-6 (ATL 1) was positive by 4 h. Another two cases (ATL 3 and 4) showed slightly higher IL-6 levels than controls at 8 and 24 h, but the last two cases (ATL 5 and 7) did not show detectable levels of IL-6 even at 24 h. It is known that IL-6 mRNA is induced in monocytes and T-cells within 5 h and 24–48 h after culture initiation, respectively (Kishimoto, 1989). Considering the high purity of ATL cells used in the study (Table I) and early detection of IL-6 at 4 or 8 h of culture, the large amount of IL-6 detected in ATL-CMs seemed to be secreted by ATL cells themselves. However, normal T cells contaminated in the T cell preparations, if activated, could have contributed some of the IL-6 that was secreted into their cultures.

IL-6 promotes the growth of PHA-stimulated thymocytes and peripheral T-cells (Kishimoto, 1989). To examine whether the IL-6 enhances proliferation of ATL-cells, ATL from patients 1, 2, 5, 6 and 7 were incubated in the presence of IL-6 (20 ng ml⁻¹) or anti-IL-6 antibody (200 u ml⁻¹) for 72 h; proliferation was measured by ³H-thymidine (³H-TdR) incorporation in the last 16 h of culture. The ³H-TdR uptake into ATL-cells was not influenced by either IL-6 or anti-IL-6 antibody (Table II).

Thus, we have demonstrated that ATL cells from four out of six patients secreted IL-6 spontaneously in vitro and that IL-6 was not detected in the sera of ATL patients in different phases of the disease. HTLV-I infection activates genes for

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Table I Surface phenotypes of the mononuclear cells from ATL patients

| Patients | CD2 | CD3 | CD4 | CD8 | CD16 | CD20 | CD25 |
|----------|-----|-----|-----|-----|------|------|------|
| ATL 1    | NTb | 42.3| 85.6| 5.7 | NT   | NT   | 20.4 |
| 2        | 99.9| 99.2| 99.6| 7.7 | 0.7  | 0.0  | 78.1 |
| 3        | 99.6| 96.4| 91.9| 6.4 | 4.1  | 0.2  | 88.6 |
| 4        | 98.1| 55  | 96.4| 1.9 | NT   | 1.3  | 94.1 |
| 5        | 89.1| 63  | 71.1| 9.2 | NT   | 9.4  | 45.2 |
| 6        | 97.7| 74.6| 88.5| 6.3 | 2.7  | 0.4  | 73.7 |
| 7        | 99.0| 29.7| 98.6| 8.0 | 0.9  | 0.8  | 76.4 |

The cells were stained by the direct or indirect immunofluorescence technique using monoclonal antibodies; OKT1, OKT3, OKT4, OKT8, OKNK1, B1 or anti-OKT1, OKT3, OKT4 monoclonal antibodies, respectively.

Table II Effect of IL-6 and anti-IL-6 antibody on proliferation of ATL-cells

| Patients | IL-6 (20 ng ml⁻¹) | Anti-IL-6 (200 μg ml⁻¹) |
|----------|-----------------|------------------------|
| None     |                 |                        |
| ATL 1    | 369 ± 24        | 361 ± 37               |
| 2        | 401 ± 6         | 434 ± 41               |
| 5        | 1880 ± 167      | 1653 ± 47              |
| 6        | 1231 ± 176      | 1291 ± 203             |
| 7        | 1559 ± 118      | 1485 ± 77              |
| normal control | 1431 ± 126 | 1383 ± 123             |

*The values are mean ± S.D.*

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