SHORT COMMUNICATION

Affinity of IL-2 receptors and proliferation of mitogen activated lymphocytes in Hodgkin's disease

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Hodgkin's disease (HD) is often described as a lymphoid disorder associated with T cell hyporesponsiveness even in the early stages of the disease (Kaplan, 1981; Kumar & Penny, 1982). Our earlier studies revealed that HD patients had normal levels of T and B cells in circulation, while they exhibited decreased mitogen (PHA) responsiveness as assessed by H-TdR incorporation as well as by T cell colony formation (Moghe et al., 1980; Mukhopadhyaya et al., 1983). Activated lymphocytes from HD patients showed reduced levels of Tac+ cells and reduced IL-2 production (Joshi et al., 1987; Mukhopadhyaya et al., 1987a). However, upon addition of exogenous IL-2, lymphocytes from about half of the HD patients showed an increase in H-TdR incorporation but none showed an increase in T cell colony formation (Mukhopadhyaya et al., 1987b). Other investigators have also reported similar abnormalities in IL-2 mediated events in T cell activation in HD (Ford et al., 1984; Souillou et al., 1985; Zamkoff et al., 1985).

The biological effect of IL-2 depends upon its binding to high affinity (HA) IL-2 receptors (IL-2R) on the membranes of activated cells, and internalisation of the complex (Fuji et al., 1986; Lowenthal & Greene, 1987; Robb & Greene, 1987). It is now well documented that IL-2R has a double chain molecular structure. The β chain (a Tac molecule) of M, 55,000 has a low affinity for IL-2 (Kd ~ 10^-13M) and displays a fast rate of association and dissociation. The α chain of M, 75,000 with intermediate affinity (Kd ~ 5 x 10^-10 to 10^-9M) has a slower rate of association and dissociation. A non-covalent association of these two makes a high affinity receptor (Kd ~ 10^-11 to 10^-10M) capable of signal transduction (Wang & Smith, 1987; Lowenthal & Greene, 1987). Although a complete Tac molecule (β chain) is the first to interact with IL-2, this interaction does not induce internalisation of the complex, a prerequisite for signal transduction.

In case of HD, the evidence gathered so far suggests that, although the proportion of Tac+ cells is less in activated lymphocyte populations in HD, the deficit is not proportional to the pronounced defect in the proliferative responses (Mukhopadhyaya et al., 1987a). Also, addition of exogenous IL-2 did not restore fully the ability of the lymphocytes to proliferate and to form colonies (Mukhopadhyaya et al., 1987b). It was therefore felt that as well as the number of Tac antigen-bearing cells per se, the number of HA IL-2R per cell (which are responsible for triggering post-binding events) may be more important in the T cell hyporesponsiveness in HD.

In the present investigations, we have therefore analysed the low and high affinity IL-2R (LA IL-2R and HA IL-2R) on PHA activated lymphocytes from the peripheral blood (PBL) of patients with HD and healthy donors, using 125I labelled recombinant IL-2 (received as a kind of gift from Cetus Corporation, USA; Doyle et al., 1985) as a ligand. Freshly diagnosed patients with HD patients, in the age group of 18–58 years (20 males and 5 females), and belonging to all stages and grades of the diseases were included in the studies. Laboratory personnel (age group 20–35 years) constituted the control group. PBL were separated on a Ficoll-Hypaque gradient, washed and cultured in DMEM (Gibco) supplemented with antibiotics and 10% human blood group AB serum. Cells (2.5 x 10^6 ml^-1) were stimulated with 0.5% PHA-M (Gibco, v/v) for 72 h at 37°C in a humidified 5% CO₂ atmosphere. The blasts thus obtained were used for further studies.

Recombinant IL-2 was labelled with 125I (Amershams, UK) by the method described by Robb et al. (1985), with some modifications. IL-2, Na125I and chloramine-T were used at the molar ratios of 1:27.8:5.1. The reaction was carried out at room temperature for 5 min and was stopped using Na₂S₂O₃ (0.278 mm). Free iodine was separated from 125I-IL-2 on a Sephadex G25 column, eluted with phosphate buffered saline containing 0.1% BSA and 1.5% acetic acid (final pH 3.9).

125I-IL-2 with specific activity above 20,000 c.p.m. ng^-1 was used for the experiments. Specificity of binding of 125I-IL-2 was tested by assessing the radioactivity bound to PHA induced blasts from PBL of two healthy donors as a function of cell number and also by determining the inhibition of binding of 125I-IL-2 by increasing amounts of unlabelled IL-2 (Figure 1). The bioactivity of 125I-IL-2 was assessed by its capacity to induce proliferation of CTLL (Gillis et al., 1978).

Proliferation of CTLL induced by 125I-IL-2 was 70–80% of that induced by unlabelled IL-2.

For experiments proper, the 125I-IL-2 binding assay was performed on PHA transformed blasts from PBL of HD patients and healthy donors, as described earlier (Fuji et al., 1986). Non-specific binding was determined in the presence of 100-fold molar excess of unlabelled IL-2 and was subtracted before transforming the binding data into Scatchard plots. Dissociation constants (Kd) and number of receptors per cell were estimated from the Scatchard plots. Wherever possible, quantitation of PHA induced blasts (using Giemsa stained cytopsin smears) and cell proliferation using H-TdR incorporation were studied simultaneously in order to compare the data with the IL-2R status.

The results indicated that lymphocytes from HD patients show significantly lower PHA induced proliferation and per cent blast formation as compared to lymphocytes from healthy donors (Table I), while, as reported earlier (Joshi et al., 1987), the percentage of Tac+ cells in PHA activated HD PBL was 42.3 ± 11.2 (P < 0.05) as compared to the value for healthy donors of 52.2 ± 14.1. The number and affinity of LA IL-2R on PHA blasts of HD patients and healthy donors were comparable (Table I). It was interesting to note that the Kd value, which indicates half the maximal concentration of the ligand (IL-2) for saturation of the receptors, was significantly higher for HA IL-2R in HD patients than for HA IL-2R in healthy donors. The requirement for higher concentration of IL-2 reflects the lower affinity of the receptors for the ligand. However, the number of receptors per cell did not differ significantly between these two groups.

In a few patients and healthy donors, where we have been able to investigate proliferation and 125I-IL-2 binding simul-
studied, 3H-TdR incorporation: net mean c.p.m. ± s.e. (no. of individuals studied, P value).

Table 1  IL-2 binding and transformation of PHA activated PBL from HD patients and healthy donors

| Parameter                          | Healthy donors | HD patients |
|------------------------------------|----------------|-------------|
| Proliferation                      | 95,133 ± 13,037 | 42,678 ± 4,651 |
| % blasts                           | 58 ± 3.6        | 43 ± 3.5    |
| Low affinity IL-2R                 |                |             |
| $K_d$ ($\times 10^{-8}$m)          | 5.67 ± 3.17     | 10.13 ± 6.37 |
| No. per cell                       | 17,018 ± 3,791  | 13,591 ± 4,502 |
| High affinity IL-2R                |                |             |
| $K_d$ ($\times 10^{-15}$m)         | 40.41 ± 7.17    | 107.59 ± 20.58 |
| No. per cell                       | 2,808 ± 280     | 2,243 ± 342  |

3H-TdR incorporation: net mean c.p.m. ± s.e. (no. of individuals studied, P value).

IL-2R ON ACTIVATED LYMPHOCYTES IN HD

Figure 1  Assessment of binding of 3H-IL-2. The top panel indicates increase in the radioactivity bound to mitogen activated lymphocytes from two healthy donors with increasing number of cells. The lower panel depicts displacement of radiolabelled IL-2 (0.5 ng) with increasing concentrations of unlabelled IL-2 in competition RIA using PHA activated lymphocytes from a healthy donor.

Figure 2  Simultaneous assessment of proliferation (3H-TdR incorporation) and affinity ($K_d$) of high affinity IL-2R on PHA activated lymphocytes from healthy donors (▲) and HD patients (●).

IL-2R ON ACTIVATED LYMPHOCYTES IN HD

Anti-Tac monoclonal antibodies have often been used to study the IL-2R status in malignancies including HD (Pizzolo et al., 1984; Joshi et al., 1987; Zamkoff et al., 1985). Radiolabelled IL-2 binding analysis, which dissects out the proportions of high and low affinity IL-2R per cell, has been performed only in the case of IL-2R expressing leukaemic cells by Uchiyama (1988) and recently by Nagel et al. (1989). The latter have studied the status of HA IL-2R on PHA activated cells from aged individuals to correlate the findings with reduced T cell responsiveness, in ageing. In the present report, we have studied the relationship of proliferation and status of HA IL-2R on mitogen activated PBL and HD.

Several factors need to be considered in explaining our data. Abnormal ratios of CD4/CD8 cells reported in HD (Romagnani et al., 1985) may influence the IL-2R status of activated PBL in this disease. However, our earlier data and those of others (Gulwani et al., 1985; Posner et al., 1981) do not show significant deviations in T cell subsets in HD. Contribution of other lymphocyte populations expressing IL-2R, such as B cells and NK cells, to the deviations in IL-2R status in HD is also uncertain. Normal proportions of B cells have previously been demonstrated in HD (Case et al., 1976; Moghe et al., 1980). Recently, we have shown that the proportion of HNK−1+ cells, which represent a subset of NK cells, does not vary between the PBL of HD patients and those from healthy donors (Rajaram et al., personal communication).

Our data have shown that activated PBL from HD have an adequate number of HA IL-2R but that they display elevated dissociation constants and a decreased proliferative response. Nagel et al. (1989) have also found decreased proliferative responses of PHA activated PBL from aged individuals, although the lymphocytes had comparable numbers of HA IL-2R and $K_d$ values to that of PBL from young individuals. They have tried to interpret their data on the basis of abnormal functions of the receptors in terms of their ability to transduce signals or failure to regulate the expression of other genes such as the transferrin receptor gene.
Our observations indicate the possibility of structural heterogeneity in HA IL-2R leading to T cell functional defects in HD. It is possible that a post-binding event such as internalisation of IL-2 and IL-2 complex leading to signal transduction (Fujii et al., 1986; Robb & Greene, 1987) may be abnormal in such situations. We are currently conducting studies on internalisation of 125I-IL-2 by activated PBL from HD in comparison with that by activated PBL from healthy donors, which may shed some light on this possibility.

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