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Defect in Lectin-induced Interleukin 2 (IL-2) Production by Peripheral Blood Lymphocytes of Patients with Hodgkin's Disease*

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Abstract—*Peripheral blood lymphocytes (PBL) of patients with Hodgkin's disease were studied for their capacity to produce interleukin 2 upon* in vitro *phytohemaglutinin stimulation in the presence or absence of either interleukin 1 or indomethacin (2 μg/ml); eight patients were studied at the discovery of their disease before receiving any therapy (onset HD; OHD). Seventeen patients were tested in long-term (>3 yr) remission (remission HD; RHD); most RHD were treated with both chemotherapy and irradiation. Fourteen healthy individuals served as controls. PBL from OHD have a significant ($P < 0.01$) defect in the production of PHA-induced IL-2. Indomethacin and IL-1 had no effect on IL-2 yield. PBL from RHD yield intermediate levels of IL-2, which are nevertheless significantly lower ($P < 0.02$) than control values. RHD recover the capacity of normal PBL to increase their production of IL-2 in indomethacin-supplemented culture medium. Interestingly, PHA responsiveness was significantly decreased only in RHD, thus not explaining the low IL-2 yield obtained in supernatants. In addition, 4-day PHA-blasts from both HD patients and control individuals increase their thymidine incorporation in the presence of purified lectin-free IL-2 to a similar degree, suggesting that their IL-2 receptors are unimpaired. Finally, OHD sera significantly inhibit PHA-induced IL-2 yield of normal PBL, suggesting that a seric component(s) may play a role in some cases. We conclude that defective IL-2 production may play a role in the well-documented deficient cellular immunity seen in Hodgkin's disease.*

INTRODUCTION

HODGKIN'S disease (HD) is a proliferative disorder of possible histiomonocytic cellular origin [1], which is associated with impairments of a wide range of T-cell dependent immune functions, such as impaired *in vivo* skin reactivity to recall antigens, depressed lectin responsiveness, impaired lymphocyte locomotion and modified T lymphocyte subsets [2-5]. This impaired T-cell function is thought to play a role in the sensitivity of patients to infectious agents and to disease progression, although this last point is still unresolved [1, 6]. The mechanism of these events is as yet poorly understood: some of these abnormalities have been attributed to the presence of circulating (non-T) suppressor cells or to numerous plasma inhibitory factors such as circulating immune-complexes, glycoproteins, glycolipids, prostaglandins, oxygenated sterols, ceruloplasmin, ferritin, zinc or copper (see [2, 3] for review).

Recently, a lymphokine secreted by T lymphocyte, interleukin 2 (IL-2), has been characterized and shown to be essential to activated T cell proliferation in immune response [7]. In experimental animal models IL-2 is able to increase the antitumoral effect of long-term cultured T lymphocytes after *in vivo* administration [8]. In man, IL-2 has been shown *in vitro* to reverse the suppressor function of cells infil-

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trating tumors or draining lymph nodes [9]. As the eventual involvement of IL-2 could explain some of the cellular abnormalities in the immune response of Hodgkin's disease, the aim of this study was to investigate the level of IL-2 production of peripheral blood lymphocytes (PBL) at various stages of Hodgkin's disease, particularly at disease discovery (before any treatment) as well as during long-term remission. In addition, the effects of interleukin 1 (IL-1) and indomethacin (IDM) on *in vitro* IL-2 production were studied and compared with healthy individuals.

MATERIALS AND METHODS

Patients

Twenty-four patients, aged 18–33, with histologically proven Hodgkin's disease were studied. Eight patients were tested at the discovery of their disease with various histological grades indicated in Fig. 1. These patients, referred to as onset HD (OHD), were tested before receiving any treatment or having laparotomy or splenectomy. In addition, 15 patients in complete remission for 3–14 yr were studied and referred to as remission HD (RHD); six of these patients were splenectomized and another had been laparotomized (all surgery performed more than 5 yr before testing). Four were treated by chemotherapy or irradiation alone and 11 received both chemotherapy and irradiation (Fig. 1). Patients under treatment were

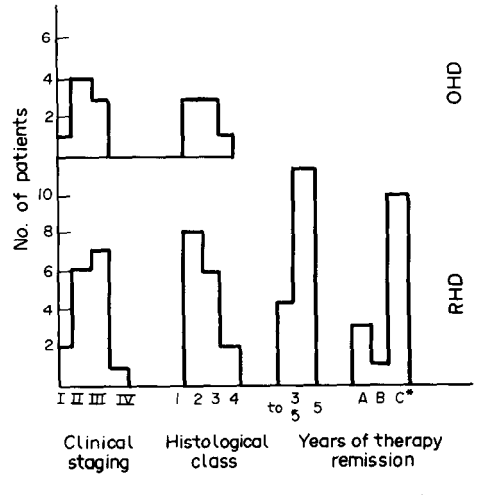

*Fig. 1. Clinical staging and histological classes of the Hodgkin's disease patients. *A, B and C in the 'therapy' columns mean respectively: chemotherapy alone, irradiation alone and chemotherapy with irradiation.*

excluded from the study to minimize variations specifically related to treatment. Fourteen age- and sex-matched healthy individuals were used as controls.

Assessment of IL-2 production by PBL of HD patients

PBL were obtained from heparinized blood by Ficoll-Hipaque sedimentation, washed twice and frozen in liquid nitrogen until used. Subsequently, thawed PBL were routinely checked for viability (>90% required) and adjusted to 8×10^6/ml in 10% human AB serum-supplemented DEM culture medium also containing fresh glutamine and gentamycin (20 μg/ml). Cells (5×10^5) were cultured in the presence of 1 μmg/ml phytohemagglutinin-P (PHA-P) (DIFCO) and 1 ng/ml of polyethylene glycol (PEG 6000) in tissue culture flasks (96 wells — 0.2 ml/well) incubated in 5% CO_2 at 37°C for 48 hr. Culture supernatants were then harvested and kept at 4°C until assayed for IL-2.

IL-2 assay

The amount of IL-2 contained in the supernatants of PHA-stimulated PBL was assayed using the IL-2 dependent CTL-L2 cell line. Briefly, CTL-L2 cells (5×10^3 in 0.2 ml/well) were incubated in Greiner tissue culture microplates in the presence of $\log_3$ dilutions of either a reference IL-2 source containing 1 unit/ml of IL-2 or the supernatants of PHA-stimulated control and HD PBL. After 48 hr of culture, 0.3 μCi of tritiated thymidine ([^{3}H]TdR) (Amersham, sp. act. 23 Ci/mM) was added to each well. The cells were harvested 12 hr later with a Skatron multisample harvester and tested for radioactivity. Results are given in units of IL-2/ml as defined previously [10].

Purified IL-2 (P-IL-2)

The lectin-free P-IL-2 used in the experiments was obtained from supernatants of PHA-stimulated lymphocytes precipitated by 80% ammonium sulfate saturation and run on an ultrogel (ACA 54) column as already described [11]. The IL-2, which eluted in the 23–24 kD fraction, was further concentrated, dialyzed against phosphate buffer saline, assayed for IL-2 activity and used at a final concentration of 5 units/ml.

PHA stimulation and response to IL-2

PBL were tested for [^{3}H]TdR incorporation after PHA stimulation in Greiner microplates (0.2 ml/well). In these experiments 1×10^5 PBL were incubated for 5 days in 0.1 ml of RPMI 1640 supplemented with 10% human AB serum and 1 μg of PHA-P (Difco). In some instances P-IL-2 was added to the PHA blast for the last 24 hr of culture.

IL-1 and indomethacin

IL-2 production of both patient and control PBL was studied in the presence and absence of exogenous IL-1. IL-1 was obtained by muramyl dipeptide (MDP) stimulation of human monocytes [12] and was kindly provided by Dr C Damais (Institut Pasteur, Paris). The IL-1-containing supernatant was free of IL-2 in our quantitative IL-2 assay. The IL-1-containing supernatant was used at a final (optimal) concentration of 10% v/v. In addition, the effect of 2 μg/ml indomethacin (ID) on IL-2 production by control and HD patients' PBL was tested under the same culture conditions used for IL-2 production since prostaglandins are involved in the regulation of IL-2 production [13].

Inhibition of IL-2 production by HD sera

PBL obtained from a normal individual were cultured with PHA following the standard procedure described above for IL-2 production. The standard human serum was replaced by test Sera (HD or normal serum of several individuals) used at final concentrations of 5 and 10% in the culture medium.

RESULTS

IL-2 production by HD PBL after PHA-stimulation (Fig. 2)

Considered as a group, HD PBL produced significantly less IL-2 ($P < 0.01$) than control PBL. The defect in IL-2 production was much more pronounced in OHD (0.59 ± 0.79 U/ml vs 3.44 ± 3.39 U/ml in controls; $P < 0.01$), in which group only two patients could produce more than 1 U/ml of IL-2. The six remaining OHD had very low (>1 U/ml) IL-2 production under lectin stimulation. Interestingly, neither exogenous IL-1 nor indomethacin addition to the culture medium could increase the IL-2 yield in these patients, indicating that the low IL-2 production was not linked to a low endogenous IL-1 activity or to PGE 2-mediated inhibition. PBL of RHD also produced significantly less IL-2 (1.42 ± 1.27 vs 3.44 ± 3.39 in controls; $P < 0.02$) but the defect was less pronounced than in OHD. IL-2 yield significantly increased in indomethacin-supplemented cultures of RHD PBL: 46 and 27% increase respectively in RHD ($P < 0.02$) and control PBL ($P < 0.01$), suggesting that prostaglandin inhibition of IL-2 production occurred in this patient subgroup as well as in control PBL. However, the IL-2 yield obtained in RHD after indomethacin supplementation of the culture medium was still significantly lower than in indomethacin-supplemented cultures of control PBL (2.64 ± 2.11 in RHD vs 4.68 ± 3.25 in controls; $P < 0.02$). In contrast, IL-1 supplementation of RHD PBL cultures did not significantly modify the IL-2 yield. Finally, no significant correlation was observed between the length of disease remission and IL-2 production of PBL, assessed either under standard conditions or in IL-1 or ID-supplemented culture medium, suggesting that low IL-2 production can persist indefinitely.

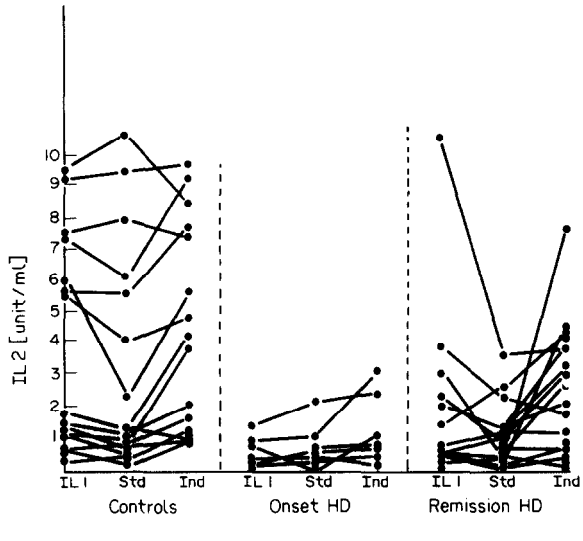

Fig. 2. PHA-induced IL-2 production by PBL of HD patients tested either at the disease discovery (OHD) or after long-term (> 3 yr) remission (RHD). Both OHD and RHD PBL produced significantly less IL-2 (respectively $P < 0.01$ and $P < 0.02$) than control individuals, PBL tested in standard (Std) conditions. IL-1 medium-supplementation did not modify IL-2 yields. IL-2 levels obtained from indomethacin (Ind)-supplemented cultures were significantly higher than those obtained in standard conditions ($P < 0.05$) in control and RHD groups.

Spontaneous and lectin-induced [^{3}H]TdR incorporation in controls and HD patients

HD PBL cultured for 5 days in the presence of P-PHA exhibited decreased thymidine incorporation as compared to control PBL, but the difference was significant only in patients with long-term remission ($P < 0.05$; Fig. 3). Late addition of P-IL-2 increased thymidine incorporation of control and HD PBL to a similar extent.

Lectin responsiveness was only slightly decreased in OHD patients and this abnormality cannot fully explain the low yield of IL-2.

Too few patients in the various clinical and histological subgroups were tested to permit possible conclusions about IL-2 production at different stages of HD. However, the lowest IL-2 levels were seen in early, low-grade disease, suggesting that defective IL-2 production may be a prominent feature at the very onset of HD.

Effect of HD patient sera on the lectin-induced production of normal PBL

As previously mentioned, several serum com-

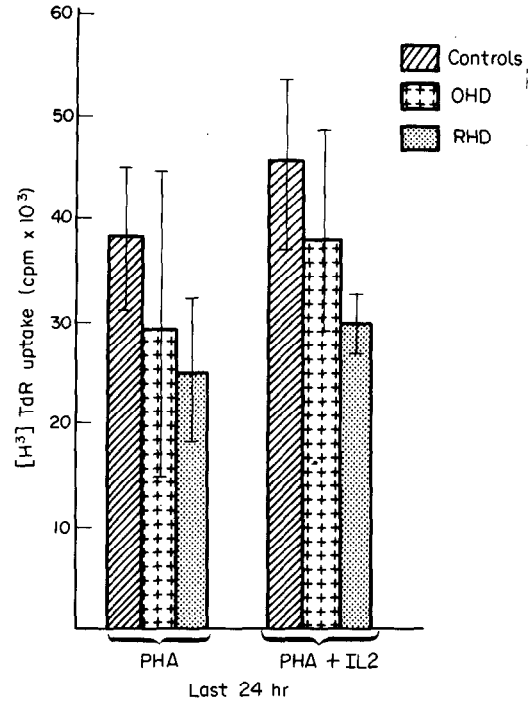

Fig. 3. Left side: PHA-responsiveness of PBL from control individuals and OHD and RHD patients. PBL of RHD have lower PHA responsiveness (P < 0.05) than controls PBL. Right side: effect of purified (lectin-free) IL-2 on [³H]TdR incorporation of 4-day PHA-blasts from controls, OHD and RHD. The average % thymidine incorporation increases are similar between the three groups (compare culumns of the left and the right sides of the figure for the control, OHD and RHD groups).

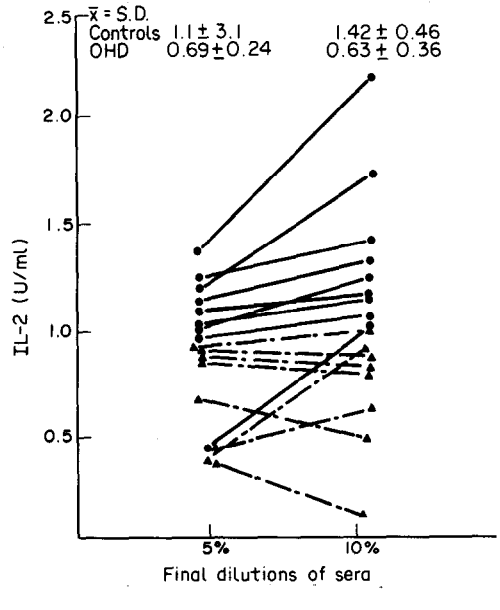

Fig. 4. IL-2 yields of PHA-stimulated normal PBL incubated with 5 and 10% dilutions of sera obtained either from OHD patients (●——●) or from healthy blood donors (▲- - -▲). The difference between the two groups was significant (P < 0.01) at both 5 and 10% of final serum concentrations.

ponents with various lymphocyte inhibitory properties have been described in HD patients [2, 3]; we thus tested the effect of incubation of normal lymphocytes with the sera of OHD patients (5 and 10% final concentration) on lectin-induced IL-2 production. Figure 4 shows that HD sera could variably inhibit the IL-2 yield of normal lymphocytes as compared to the several 'random' sera from healthy individuals. This inhibitory effect was mainly observed at a high serum concentration (10%). Although sera of normal individuals support IL-2 production of normal cells to a variable extent, Fig. 2 shows that the IL-2 yield of normal PBL was nevertheless significantly decreased when HD sera were used in the culture medium instead of control sera ($P < 0.01$).

DISCUSSION

Our results show for the first time that there is a profound defect of lymphocyte PHA-induced IL-2 production in patients with Hodgkin's disease. This defect is particularly pronounced at the time of disease onset. PBL of patients in long-term remission still show abnormal IL-2 production which never reverts to control values, even after more than 2 yr. Although the number of OHD available is relatively small, it seems that the low IL-2 production was found in patients with non-invasive disease of low histological grade as well as in patients with more advanced disease at the time of disease discovery. Although HD PBL are slightly less responsive to P-PHA than control PBL, this does not fully account for the low yield of IL-2, particularly in the case of OHD lymphocytes. The role of an increase of monocyte percentage in OHD or RHD has been ruled out since their values were not significantly different (respectively 6.7 ± 2.2 and 6.5 ± 3.7) from the values in normal controls (6 ± 2). Increased prostaglandin inhibition [13] does not appear to be a factor as indomethacin has no effect on the IL-2 production of OHD PBL. Furthermore, exogenous IL-1 did not modify IL-2 production, indicating that this monokine [14] was not lacking in test cultures nor was IL-1 seemingly inhibited as described recently in certain inflammatory diseases [15]. Hodgkin's disease patients' sera have been found to exhibit inhibitory properties on lymphocytes [2, 3]. PHA-induced IL-2 production of normal lymphocytes cultured in the presence of various concentration of OHD sera suggest that, at 10% final concentration, OHD sera inhibit also lymphokine production as compared to different normal individuals' sera.

Although direct binding of labelled IL-2 on HD PBL would be required to precisely analyze a possible abnormality of IL-2 receptors, addition

of purified (lectin-free) IL-2 [11] to 5-day PHA-stimulated PBL increased the [^{3}H]TdR incorporation of the HD cells to the same extent as in normal PBL. This suggests that there are functional IL-2 receptors expressed at the surface of activated HD PBL.

PBL of patients with long-term disease remission (>3 yr) have significantly decreased lectin responsiveness and produced lower amounts of IL-2 than PBL of normal individuals; however, this low IL-2 production still exceeds that of OHD patients. In addition, as opposed to the PBL of OHD, normal and RHD PBL increase their IL-2 yield when exposed to indomethacin, suggesting that in this group monocytes may, as in normal individuals, control IL-2 production via prostaglandin secretion since this last cell is the only cell to produce this factor in PBL [13].

Whether or not the defect in IL-2 production is a constant feature in Hodgkin's disease is impossible to assess, especially since HD patients received chemo- and radiotherapy, which in itself can depress T cell functioning for prolonged periods [6]. Several reports have claimed that low T4/T8 ratios are found in HD patients in remission [16]. As IL-2 is predominantly produced by $T4^+$ cells one can also hypothesize that our findings may be related to this inverted ratio in RHD.

Whatever the mechanism of the IL-2 defect in HD, our data raise questions about both the etiologic role of defective IL-2 production (i.e. defect of IL-2 preceding the disease) and its influence on the course of the disease.

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