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Interleukin-2 activity in chronic active liver diseases: response by T cells and in the autologous mixed lymphocyte reaction

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SUMMARY

The T cell growth factor, interleukin-2 (IL-2), is a lymphokine which supports the immunoregulatory function of T cells. We measured the production of and response to IL-2 of peripheral blood T cell subsets from patients with chronic active liver diseases (CALD) and other liver diseases (Others) by the proliferative response of the cells activated with phytohaemogglutin P. Both production of and response to IL-2 of T cells from 24 patients with CALD were markedly decreased ($P < 0{\cdot}001$) in comparison with 13 controls. T cells from 10 patients with Others yielded low IL-2 titre ($P < 0{\cdot}05$) and responded to IL-2 in a depressed manner ($P < 0{\cdot}05$). $OKT4^+$ and $OKT8^+$ cells from five CALD patients as well as five controls equally produced IL-2 and responded to it. However, IL-2 production ($P < 0{\cdot}05$) and response to IL-2 ($P < 0{\cdot}01$) of $OKT4^+$ cells from CALD patients were decreased in contrast to those of $OKT8^+$ cells. We also examined the effect of IL-2 on the autologous mixed lymphocyte reaction. A highly significant increase ($P < 0{\cdot}001$) in the proliferative response of $OKT8^+$ cells and unseparated T cells from 15 patients with CALD occurred with the addition of IL-2 although the values were still lower ($P < 0{\cdot}01$) than those of $OKT8^+$ and unseparated T cells from 12 controls. Addition of IL-2 did not result in a significant increase of the reactivity of $OKT4^+$ cells from patients with CALD. These results further delineate the nature of the immunoregulatory aberration in CALD.

Keywords interleukin-2 T cell subsets autologous mixed lymphocyte reaction chronic active liver diseases

INTRODUCTION

The T cell growth factor interleukin-2 (IL-2) is a non-specific, genetically unrestricted soluble factor produced by T cells stimulated with antigens, mitogens or alloantigens (Watson & Mochizuki, 1980; Smith, 1980). Recent studies indicate that IL-2 is an important lymphokine in supporting the immunoregulatory functions of T lymphocytes (Kern *et al.*, 1981; Farrar *et al.*, 1982).

Our previous studies demonstrated that immunoregulatory T cell function is substantially reduced in some patients with chronic active liver diseases (CALD), suggesting that the immunoregulatory mechanism of host defence may be important in the inhibititon of liver cell injury in CALD (Kakumu, Yata & Kashio, 1980). The production of and response to IL-2 have not been studied in patients with CALD.

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The T cell proliferative response to autologous non-T cells is termed the autologous mixed lymphocyte reaction (AMLR) and this reaction reflects self regulation of immune responses (Opelz *et al.*, 1975). Several studies have shown that the AMLR is decreased in autoimmune disease (Sakane, Steinberg & Green, 1978; Miyasaka *et al.*, 1980; James *et al.*, 1980). In this study we have investigated T cell subsets with regard to their ability to produce IL-2 and respond to it in CALD. We also examined the effect of the addition of IL-2 on the AMLR.

MATERIALS AND METHODS

Patients. We studied 24 patients with CALD (age range 28–60 years; mean 45 years), 10 patients with other liver diseases (Others) (age range 26–54 years; mean 41 years) including chronic persistent hepatitis (five cases), inactive cirrhosis (three cases) and alcoholic fatty liver (two cases). Thirteen healthy volunteers, matched with the patients for age and sex, served as control subjects. The diagnosis of CALD was based on biochemical evidence of liver injury and hepatic histology according to the criteria of an International Study Group (Leevy, Popper & Sherlock, 1979). In the CALD specimens, all patients had round cell infiltration involving portal tracts and moderate to severe piecemeal necrosis of liver cells extending outward into the parenchyma from the limiting plate could be detected. On the other hand, in patients with chronic persistent hepatitis and cirrhosis inflammatory round cell infiltration of portal tracts was less pronounced with minimal or absent piecemeal necrosis of the limiting plates. Each biopsy was assessed for the architecture (normal, multilobular collapse with bridging hepatic necrosis, fibrosis and cirrhosis), graded (absent = 0, minimal = 1, moderate = 2 and severe = 3) for each of seven histological features (lymphocyte and plasma cell portal tract infiltrate, piecemeal necrosis, spotty lobular necrosis, bile duct reduplication, cholestasis, Kupffer cell activity and presence of fat) and a total biospy score obtained. In addition, a separate score was included for the observer's overall subjective assessment of inflammatory activity (minimal = 1, moderate = 2 and severe = 3). Eight of 24 patients with CALD had liver cirrhosis, and four were serum HBsAg positive. Three of 10 patients with Others were HBsAg positive in their serum. Since corticosteroids may affect IL-2 production (Gillis, Crabtree & Smith, 1979), we selected patients not on steroid treatment during the course of study.

Preparation of cells. Peripheral blood mononuclear cells (PBM) were isolated from heparinized venous blood by Ficoll-Hypaque gradient sedimentation. The interphase PBM were suspended at 4×10^6 cells/ml in RPMI 1640 (GIBCO) culture medium (RPMI 1640 supplemented with 10% heat-inactivated fetal bovine serum [FBS], glutamine and antibiotics). Monocytes were partially depleted by incubating PBM on glass Petri dishes for 60 min at 37°C in a moist atmosphere of 5% CO_2 (Kakumu *et al.*, 1978). The non-adherent cells were rosetted with neuraminidase treated sheep erythrocytes (E). The E rosetted cells and non-rosetted cells were separated by centrifugation on a Ficoll-Hypaque density gradient. Of the rosette forming cells, designated T cells, over 95% formed rosette with E. Of the interphase cells, designated B cells, less than 3% formed resette with E.

Monoclonal antibodies (MoAbs). The hybridoma derived MoAbs OKT4 and OKT8, were purchased from the Ortho Pharmaceutical Corporation. OKT4 reacts with 50–60% of adult peripheral T cells and OKT8 with 20–30% of the cells (Reinherz & Schlossman, 1980). $OKT4^+$ defines the inducer/helper subset (Reinherz *et al.*, 1979) and $OKT8^+$ the suppressor/cytotoxic population (Reinherz *et al.*, 1980).

Isolation of T cell subsets. To isolate $OKT4^+$ cell rich and $OKT8^+$ cell rich populations, unfractionated T cells were suspended at 5×10^6 cells/ml in RPMI culture medium and OKT4 or OKT8 were added to cell suspensions at a final dilution of 1:150. After 60 min incubation at 37°C, rabbit sera as a source of complement were added to the cell suspensions at a final dilution of 1:4, and further incubation was performed at 37°C for 60 min. After washing, the cells were suspended in RPMI 1640 culture medium. Analysis of the resultant populations by indirect immunofluorescence microscopy with fluorescein labelled goat anti-mouse IgG (Cappel Laboratories) showed that the OKT4 treated population contained 84–93% $OKT8^+$ cells, and the OKT8 treated population contained 87–94% $OKT4^+$ cells. OKT4 treated cells were used as the $OKT8^+$ and OKT8 treated cells as the $OKT4^+$ cells.

Production of IL-2. An aliquot (1 ml) of T cells or T cell subsets containing 2×10^6 cells was cultured for 48 h in RPMI with 5% FBS and 1 μg phytohaemagglutinin P (PHA-P) (DIFCO Laboratories) in 12×75 mm culture tubes in a 5% CO_2, 100% humidity, 37°C atmosphere. After culture, the tubes were centrifuged to collect the supernatants, and these were stored at −20°C.

Assay for IL-2 activity. An aliquot (1 ml) of cells from a normal subject containing 1×10^6 cells was incubated for 5 days in RPMI culture medium with 1 μg PHA-P at 37°C in a 5% CO_2 atmosphere. After washing, the cells were suspended at 1×10^6 cells/ml in RPMI culture medium and cultured with 20% lectin free IL-2 (Associated Biomedic Systems) at 37°C in a 5% CO_2 atmosphere. After 3–4 days culture, the cells were resuspended at 1×10^6 cells and cultured with IL-2 in culture medium. The cells were used 3 days after the third passage. They were washed in RPMI and placed in microculture plates (10^4 cells/well) in 100 μl of RPMI with 5% FBS. The supernatants being tested were added at three different dilutions, and the plates were incubated for 72 h at 37°C. For the last 20 h of incubation, 0·5 μCi of ^{3}H-thymidine (New England Nuclear) was added to each well, and thymidine incorporation was measured by a liquid scinitillation counter. Data were expressed as the average of the counts per minute (ct/min) of quadruplicate cultures.

Response of cells to IL-2. To test the response of T cells or T cell subsets from controls or patients to IL-2, the cells (1×10^6 cells in 1 ml) were cultured for 5 days with 1 μg PHA-P. After washing, 10^4 cells in 100 μl RPMI with 5% FBS were placed in microculture plates and incubated for 72 h at 37°C in a 5% CO_2 atmosphere with 1:3 diluted IL-2. The time and dose of ^{3}H-thymidine and counting procedures were as detailed above.

AMLR. The AMLR was set up by culturing T cells ($1{\cdot}5 \times 10^5$ cells/well) and an equal number of irradiated (2,500 rad) stimulating B cells in microculture plates in a final volume of 200 μl in RPMI culture medium. Cultures were incubated for 6 days at 37°C in a moist atmosphere of 5% CO_2. Addition of ^{3}H-thymidine and counting procedures were done in an identical way to the above.

Statistical analysis. All data were analysed using Student's *t*-test.

RESULTS

Production of IL-2

As shown in Fig. 1, T cells from 22 patients with CALD produced significantly less IL-2 with the

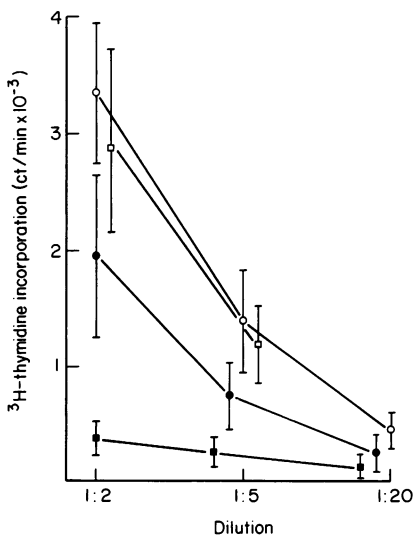

Fig. 1. Proliferative response induced on IL-2-dependent T cells by three different dilutions of the supernatants of PHA-P activated T cells from normal subjects (○——○), patients with Others (□——□) and patients with CALD (●——●). The response to the supernatant of normal non-activated T cells is also shown (■——■).

averages of 1,966±891 (±s.d.) ($P<0.001$) and 789±302 ct/min ($P<0.001$), respectively, in comparison with those from normal subjects (3,350±712 and 1,389±523 ct/min, respectively) at 1:2 and 1:5 dilutions of culture supernatants, while T cells from nine patients with Others produced slightly less IL-2 (2,916±829 ct/min, $P<0.05$) at 1:2 dilution. Preliminary experiments showed that PHA-P concentration used in this study and left in the supernatants did not influence on production of IL-2.

Twelve of 22 patients with CALD and one of nine patients with Others produced less IL-2 than the mean − 2 s.d. of that produced by cells from normal subjects at 1:2 dilution of the supernatants (Fig. 2).

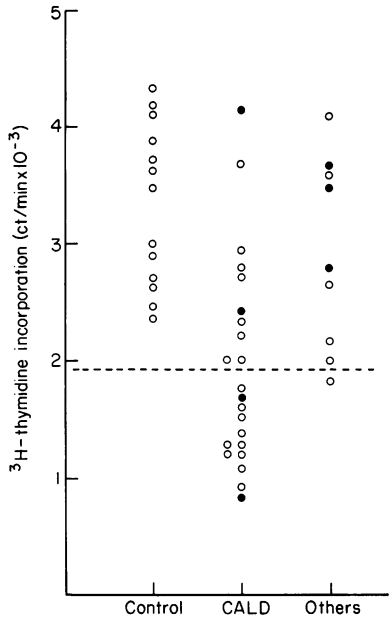

Fig. 2. Proliferative response induced on IL-2-dependent T cells by the supernatants (1:2 dilution) of activated T cells. Dotted line indicates mean − 2 s.d. of normal subjects. ○ = HBsAg negative; ● = HBsAg positive.

As shown in Fig. 3, OKT4$^+$ cells tended to produce more amounts of IL-2 than OKT8$^+$ cells in control in contrast to patients with CALD although the differences were not significant respectively in both controls and CALD. OKT4$^+$ cells of five patients with CALD produced less IL-2 (1,727±637 ct/min, $P<0.05$) compared with controls (3,442±1,056 ct/min).

Response to IL-2

The response of T cells to IL-2 in 24 patients with CALD was lower with an average of 15,838±7,493 ct/min ($P<0.001$) than that of T cells from controls (26,524±5,931 ct/min) when activated with PHA-P (Fig. 4). The response of T cells from 10 patients with Others was also slightly decresed (20,668±5,166 ct/min, $P<0.05$). In 13 patients with CALD and one patient with Others the values were below the normal range. Somewhat higher response to IL-2 by non-stimulated T cells from patients with CALD (713±250 ct/min) than by those from normal subjects (585±242 ct/min) was noticed.

OKT8$^+$ cells tended to respond greater to IL-2 than OKT4$^+$ cells in both controls and patients with CALD although significant differences were not found respectively. The response of OKT4$^+$ cells of five patients with CALD was decreased (11,530±3,185 ct/min, $P<0.01$) in comparison with that of controls (21,920±5,755 ct/min), whereas there was no significant difference in the response of OKT8$^+$ between controls and patients with CALD (Fig. 5).

In patients with CALD, the degree of IL-2 production or response to IL-2 did not correlate with

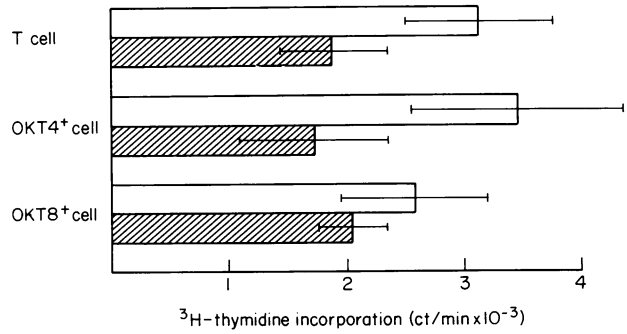

Fig. 3. proliferative response induced on IL-2-dependent T cells by the supernatants (1 : 2 diltuion) of activated T cell subsets from normal subjects (□) and patients with CALD (■). Mean ± s.d. is shown by horizontal lines.

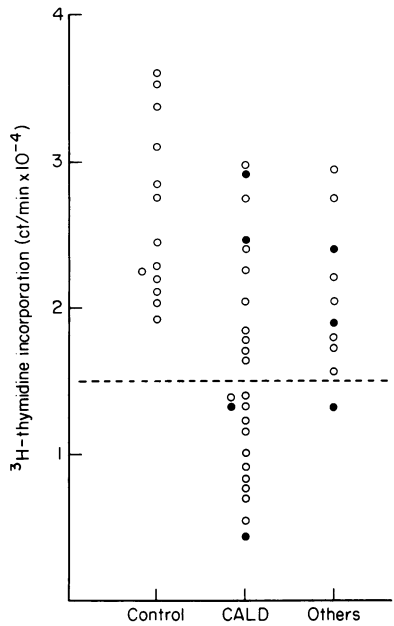

Fig. 4. Proliferative response of PHA-P activated T cells from normal subjects, patients with CALD or Others to IL-2. Dotted line indicates mean − 2 s.d. of normal subjects. ○ = HBsAg negative; ● = HBsAg positive.

age, levels of serum total bilirubin, glutamate pyruvate transaminase, gamma globulin, and HBsAg or anti-HBs. However, on review of the liver biopsy histology, severity of piecemeal necrosis of liver cells tended to vary inversely with the amounts of IL-2 production and response to IL-2.

Effect of IL-2 on AMLR

The mean value for the AMLR in 15 patients with CALD, 11,168 ± 6,564 ct/min, was lower ($P < 0{\cdot}001$) than that observed in 12 normal individuals, 23,696 ± 7,997 ct/min. In controls, when OKT4$^+$ cells were incubated with autologous B cells, their AMLR activity (20,160 ± 5,573 ct/min) was comparable to that seen in unseparated T cells. On the other hand, OKT8$^+$ cells proliferated only minimally in the AMLR (5,042 ± 1,079 ct/min, $P < 0{\cdot}001$). The similar, but decreased reactivities of OKT4$^+$ (9,870 ± 5,823 ct/min, $P < 0{\cdot}001$) and OKT8$^+$ (2,800 ± 1,567 ct/min, $P < 0{\cdot}01$) cells from patients with CALD were demonstrated compared to those of OKT4$^+$ and OKT8$^+$ cells from controls (Fig. 6).

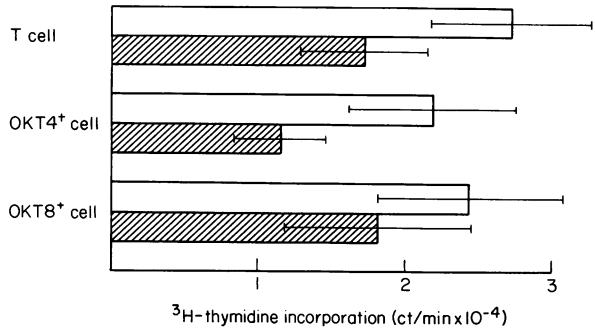

Fig. 5. Proliferative response of activated T cells from normal subjects (□) and patients with CALD (■) to IL-2. Mean ± s.d. is shown by horizontal lines.

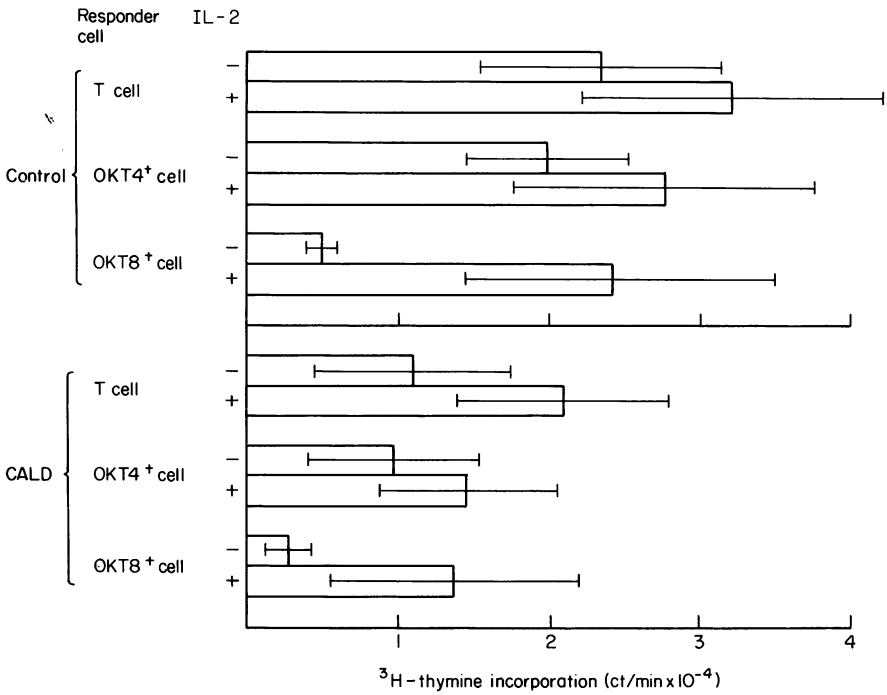

Fig. 6. Effect of the addition of IL-2 on the responder cells in the AMLR. Mean ± s.d. is shown by horizontal lines.

When IL-2 was added to the control AMLR culture, the proliferative response of $OKT8^+$ cells were highly increased (24,266 ± 9,185 ct/min, $P < 0.001$) in comparison with the AMLR in the absence of IL-2, while the addition of IL-2 resulted in a slight increase of the reactivity of $OKT4^+$ (27,613 ± 8,192 ct/min, $P < 0.05$) and unseparated T cells (32,293 ± 10.256 ct/min, $P < 0.05$). In patients with CALD, a highly significant increase in proliferation of $OKT8^+$ (13,687 ± 8,135 ct/min, $P < 0.001$) and unseparated T cells (21,168 ± 6,994 ct/min, $P < 0.001$) was observed by the addition of IL-2 when compared with the culture without IL-2, although the values were still lower than those of $OKT8^+$ ($P < 0.001$) and unseparated T cells ($P < 0.01$) from controls. In contrast, $OKT4^+$ cells from patients with CALD did not show significant increase of the reactivity in the presence of IL-2.

DISCUSSION

IL-2 is known to support T cell proliferation under mitogenic (Paetkau *et al.*, 1980) or antigenic stimuli (Watson & Mochizuki, 1980) and in the AMLR test (Smolen *et al.*, 1981). It also contributes to the generation of cytotoxic T cells (Kern *et al.*, 1981), to the abrogation of the suppressor cell function induced by concanavalin A (Palacios & Möller, 1981) and to the promotion of helper cell function (Kern *et al.*, 1981). These functions have been found to be altered in CALD (Warnatz *et al.*, 1979; Kashio, Hotta & Kakumu, 1981; Holdstock, Ershler & Krawitt, 1981). In addition, patients with systemic lupus erythematosus have been recently shown to have a similar defect in the response to and production of IL-2 (Alcocer-Varela & Alarcon-Segovia, 1982). Thus IL-2 may be one of the most important humoral factors in the regulation of cellular immunity.

Our present data indicate that production of and response to IL-2 by T cells from patients with CALD is greatly decreased, and $OKT4^+$ cells are responsible for those abnormal findings. We also demonstrated that $OKT8^+$ as well as $OKT4^+$ cells are able to produce IL-2, and respond to IL-2 as indicated by others (Luger *et al.*, 1982). These results suggest that the decreased AMLR value in CALD may be, at least at part, due to deficiency in IL-2 production and response to IL-2 by $OKT4^+$ cells. Significant levels of IL-2 are secreted during the AMLR culture and such IL-2 can augment the AMLR activity (Smolen *et al.*, 1981; Luger *et al.*, 1982; Alcocer-Varela & Alarcon-Segovia, 1982). We similarly showed that the addition of IL-2 improved the proliferative response of unseparated T and $OKT8^+$ cells for the AMLR in patients with CALD.

Smith & Talal (1982) have suggested that if the AMLR is normal and IL-2 is produced, then cytotoxic cells are generated that can clear the body of cells with surface determinants altered by virus or by environmental chemicals. Normal AMLR also leads to intact suppressor pathways, either because of direct generation or because of activation by T helper inducer cells. Thus, immunoregulation and immunological homeostasis are maintained. If the AMLR and IL-2 production are deficient, then both persistence of altered cells and disordered immunoregulation could result. By this pathyway, autoimmune disease could result from a humoral response to the persisting altered cells and cross-reactions of the antibodies on normal cell constituents.

The potential reversibility of defective AMLR and deficient IL-2 production suggest the possibility that restoration of these defects to normal may be a clinically useful therapeutic approach. Further work should be directed at defining the precise mechanism responsible for the AMLR and IL-2 defects and at therapy that will restore these two critical aspects of immunoregulatory activity to normal levels. Moreover, we have not yet determined whether a primary defect of T cells or a secondary one, due to some factors such as autoantibody, contributes to the decreased IL-2 production and response to it.

Finally we can not formally exclude the possibility that macrophages may have contributed to the activation of lymphocytes by the polyclonal stimuli in this study. The fact that a secretory product of macrophages, namely interleukin-1 (IL-1), can further increase IL-2 secretion by T cells points out the importance of this augmenting signal produced by macrophages (Luger *et al.*, 1982). However we have not investigated the relationship between IL-1 and IL-2 in patients with CALD. This interaction remains to be clarified.

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