

Presence of preactivated T cells in hemodialyzed patients: Their possible role in altered immunity

(hemodialysis/interleukin 2/B-cell growth factors/activated T cells)

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ABSTRACT Interleukin 2 (IL-2) and B-cell growth factors I and II (BCGF I and BCGF II) are lymphokines produced by T cells that play a major role in T- and B-cell cooperation. Peripheral blood lymphocytes from 12 uremic patients undergoing intermittent hemodialysis were tested for their capacity to produce IL-2 and BCGFs and to respond to these soluble mediators. IL-2 and BCGF activities were determined by means of two biological assays (proliferation of IL-2-dependent cytotoxic T-cell line CTLL-2 and of anti-human IgM (μ chain)-stimulated normal B cells, respectively) in the supernatants of phytohemagglutinin A-stimulated T-cell cultures. IL-2 activity was significantly decreased in patients as compared to normal controls (mean \pm SEM, 0.28 ± 0.09 unit per ml) in hemodialyzed patients versus 1.02 ± 0.16 units per ml in normal controls). This profound abnormality contrasted with the normal activity of the BCGFs that was invariably observed in the same supernatants. A similar dissociation was detected when analyzing the sensitivity of uremic B and T cells to exogenous purified lymphokines. Anti-IgM (μ chain)-stimulated uremic B cells exhibited a normal response to recombinant IL-2 and to chromatography-purified BCGF I and BCGF II. Resting B cells did not show any increased reactivity to these lymphokines. In contrast, whereas in normal controls recombinant IL-2 exclusively induced the proliferation of T cells that had been previously activated by a mitogen, resting T cells from uremic patients were highly responsive to exogenous IL-2. This abnormal response was paralleled by significantly increased proportions of peripheral T cells recognized by the anti-Tac monoclonal antibody that specifically binds to the IL-2 receptor. These data clearly show the existence in hemodialyzed patients of abnormally high proportions of T cells presenting phenotypic and functional signs of preactivation. This increased T-cell IL-2 receptor expression may offer an explanation to the deficient IL-2 activity observed in patients' supernatants (by inducing increased absorption of the lymphokine). The potential relevance of these preactivated T cells to the depressed cell-mediated immunity observed in hemodialyzed patients is outlined.

Patients undergoing intermittent hemodialysis present a depressed *in vivo* and *in vitro* immune reactivity that is independent of their initial pathology (1). The immunopathological basis of this immunosuppression is far from being entirely understood. Hemodialyzed patients show a decrease of absolute numbers of circulating lymphocytes, cutaneous anergy, prolonged survival of skin grafts (2, 3), and abnormally low antibody responses to some (but not all) thymus-dependent antigens like hepatitis B vaccine (4). In contrast, a normal response against thymus-independent antigens like

pneumococcal vaccine is generally observed. That all of these immune aberrations may not be simply ascribed to the lymphocytopenia is indicated by several reports showing that the purified lymphocytes from patients exhibit low *in vitro* responses to alloantigens and in some cases to mitogens (5).

In this work, one of the main factors controlling the level of immune responses has been studied in hemodialyzed patients—that is the production and targeting of two major lymphokine activities involved in lymphocyte cooperation: interleukin 2 (IL-2) and B-cell growth factors (BCGFs). These soluble mediators are produced by lymphocytes following antigenic or mitogenic stimulation and promote the proliferation and/or the differentiation of functionally distinct T- and B-lymphocyte subsets (6–8). In addition to evaluations of IL-2 and BCGF synthesis by T cells from patients, the response of T and B lymphocytes to the exogenous purified or semipurified lymphokines (recombinant IL-2 and chromatography-purified BCGF I and BCGF II) was tested. Moreover, the anti-Tac monoclonal antibody was used to quantitate patients' circulating T cells that expressed the IL-2 receptor, which is considered an activation marker (9). The results obtained show the existence in hemodialyzed patients of abnormally high proportions of circulating T cells presenting phenotypic and functional signs of preactivation. The potential immunopathological significance of this observation will be outlined.

PATIENTS AND METHODS

Patients. Twelve adult patients (six men and six women), stabilized on renal replacement therapy (at least 3 months of intermittent hemodialysis treatment prior to the study) were selected for the study. They ranged in age from 28 and 64 years (mean, 40.5 ± 3.8). In these patients, chronic renal failure was due to chronic glomerulonephritis (five cases), chronic interstitial nephropathy (two cases), nephronophthisis (one case), secondary amyloidosis (one case), nephroangiosclerosis (one case), or an undetermined cause (two cases). Patients underwent hemodialysis twice a week for 4–5 hr using Cuprophan (Hospal, Lyon, France) or polyacrylonitrile membranes and water pretreated by filtration. Patients included in this trial did not receive treatments known to interfere with the immune system (such as corticoids, anti-lymphocyte antiserum, etc.).

Three patients presented circulating hepatitis B surface antigen (MON, HAM, and AND), and four had significant titers of antibodies against hepatitis B surface antigen (CHI, SAM, MAG, and JMA). Four patients had been vaccinated

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Abbreviations: IL-2, interleukin 2; BCGF, B-cell growth factor; PBM, peripheral blood mononuclear cells; PHA, phytohemagglutinin A; SRBC, sheep erythrocytes; LGL, large granular lymphocytes.

against hepatitis B virus; only one of them developed protective antibody titers (HAD), whereas the other three (TUR, CHA, and DAM) showed no response. All patients were negative by Elisa for human immunodeficiency virus HIV. All blood samples were collected before dialysis and twice at 1-month intervals to test the reproducibility of the assays used. Control samples were obtained from 27 age- and sex-matched healthy subjects.

Cellular Preparations. Peripheral blood mononuclear cells (PBM) were isolated by centrifugation at $400 \times g$ on a Ficoll-Paque (Pharmacia, Uppsala, Sweden) gradient. PBM were recovered at the interface and washed twice in Hanks' balanced salt solution (Eurobio, Paris, France). When required, PBM were separated into T cells and non-T-cells by rosetting with neuraminidase (Behringwerke)-treated sheep erythrocytes (SRBC). The rosetting erythrocyte E^+ cells thus obtained contained 92–95% OKT3⁺ T cells, and the nonrosetting E^- non-T-cell population contained 2–5% OKT3⁺ cells, as assessed by indirect immunofluorescence (described below).

When needed, B lymphocytes contained in the E^- population were purified by means of an additional E-rosetting procedure, adherence to plastic to partially eliminate contaminating monocytes (1 hr of incubation on culture Petri dishes), and finally treatment with the Leu-1 monoclonal antibody (Becton-Dickinson) and rabbit complement. These preparations (referred to as enriched B cells) contained 80–85% surface Ig-expressing cells, 5–10% peroxidase-positive cells, and <0.5% Leu-1⁺ and/or OKT3⁺ cells. When cultured in the presence of mitogenic phytohemagglutinin (PHA) concentrations, these preparations were unable to proliferate. Furthermore, after a 4-day stimulation with PHA or growth factors, this B-cell-enriched population still showed <0.5% Leu-1⁺ and/or OKT3⁺ cells.

Immunofluorescence Test. Indirect immunofluorescence was performed on PBM, E^+ , and E^- cells with the monoclonal antibodies UCHT1 (given by P. Beverley), OKT3, OKT4, and OKT8 (Ortho Pharmaceutical) that respectively react with peripheral mature human T cells, helper/inducer T cells, and cytotoxic/suppressor T cells (10). In some patients, the anti-Tac monoclonal antibody (given by T. Waldmann), which specifically recognizes the IL-2 receptor, was used (9). Similarly, in some experiments the monoclonal antibody 3B8, which recognizes human large granular lymphocytes (LGL), was used (11). The indirect immunofluorescence technique has been described in detail (12). Briefly, the cells ($10\text{--}20 \times 10^6$ per ml) were incubated at 4°C with appropriate dilutions of OKT3, OKT4, OKT8, anti-Tac, or 3B8 antibodies. After two washes in cold Hanks' balanced salt solution containing 5% fetal calf serum and 0.2% sodium azide, the cells were labeled for 30 min at 4°C with fluorescein-conjugated goat anti-mouse IgG antiserum (Nordic, Tilburg, The Netherlands). Following a washing procedure and resuspension, one drop was examined under Leitz Dialux microscope equipped for epifluorescence, and 200 cells per slide were counted. The percentage of each T-cell subset with respect to the total number of mononuclear cells counted was then calculated, and the percent OKT4/percent OKT8 cell ratio was evaluated. When necessary, the absolute counts of each separate T-cell subset were deduced by taking into account the total lymphocyte counts.

Lymphokine Production. Culture medium was RPMI 1640 (GIBCO) supplemented with penicillin (100 units per ml), streptomycin (100 $\mu\text{g}/\text{ml}$), and fetal calf serum (2%). E^+ cells were adjusted to 1×10^6 cells per ml and distributed (1 ml/well) in 24-well Limbro culture plates (GIBCO). They were then stimulated with 0.4 μg or 4 μg of PHA-P (Pharmacia) per ml. Supernatants were collected after 48 hr of culture (in humidified 95% air/5% CO₂ at 37°C), centri-

fuged, sterilized by filtration (45- μm pore size), and stored at -20°C until tested for IL-2 and BCGF activity.

IL-2 Titration. Culture supernatants were thawed and tested for their IL-2 activities on the IL-2-dependent murine cytotoxic T-cell line CTLL-2. Briefly 4×10^3 cells were cultured in round-bottom microplates in the presence of serial dilutions of the test supernatants. The proliferation was analyzed after 74 hr by means of a 4-hr [³H]thymidine (CEA, Gif-sur-Yvette, France) incorporation assay. Units were calculated by comparison with the mitogenic activity of serial dilutions of a reference supernatant containing by definition 1 unit per ml (produced by 48 hr of PHA stimulation of normal PBM) (13). The unit concentration of each supernatant was calculated by using a probit program.

Sensitivity of T Cells to Exogenous IL-2. Non-stimulated T cells. Freshly isolated E-rosetting T cells were cultured (0.5×10^6 per ml) in round-bottom microtiter culture plates (0.2 ml per well) in RPMI 1640 medium containing 10% fetal calf serum either alone or supplemented with 1 unit of recombinant IL-2 (Sandoz, Wien, Austria) per ml. Proliferation was assayed after 48 or 72 hr of culture by means of 4- and/or 16-hr thymidine incorporation.

Stimulated T cells. E-rosetting T cells (1×10^6 cells per ml of RPMI 1640 medium containing 10% fetal calf serum) were stimulated in Limbro plates (1 ml per well) with 1 μg of PHA for 4 days. The cells were recovered, washed and kept in culture in complete medium without mitogen for 24 additional hr. Then, the cells were recovered and cultured in round-bottom microtiter plates (10^4 cells per well) with complete medium containing 1 unit of recombinant IL-2 per ml. After 18 hr of culture, the proliferation was assessed by means of [³H]thymidine incorporation.

Titration of BCGFs (B-Cell Proliferating Activity). Activity of BCGFs in culture supernatants was tested by means of the capacity to induce significant proliferation of enriched B cells from normal donors stimulated with insolubilized rabbit anti-human IgM (μ chain) antibody. Normal B cells (10^5) were cultured in flat-bottom microplates in 200 μl of RPMI 1640 medium containing 1% fetal calf serum, anti-IgM (μ chain) antibody (Bio-Rad) at 4 $\mu\text{g}/\text{ml}$ (final concentration) and serial dilutions of test supernatants.

Proliferation was measured by means of [³H]thymidine incorporation during the last 16 hr of a 3-day culture. The results were expressed by the following proliferation index:

$$\frac{\text{cpm (B cells + anti-IgM } (\mu) \text{ + test supernatant)} - \text{cpm (B cell + anti-IgM } (\mu))}{\text{cpm (B cells + anti-IgM } (\mu))}$$

Sensitivity of B Cells to Exogenous BCGF I, BCGF II, and IL-2. The same sort of B-cell proliferating assay first described was used. Enriched B-cell populations of patients and controls were tested. Semipurified BCGF I was purchased from Cellular Products (Buffalo, New York) and used at 10% (vol/vol) final concentration. BCGF II (or 50-kDa BCGF) was obtained as described (14) after lectin (concanavalin A) chromatography and was used at 10% (vol/vol) final concentration. Recombinant IL-2 was used at a final concentration of 1 unit per ml.

Statistical Analysis. Statistical comparison of mean values was performed by using Student's "t" test.

RESULTS

Distribution of Peripheral Regulatory T-Cell Subsets. The percentage values of total T cells, helper/inducer T cells, and cytotoxic/suppressor T cells were analyzed by labeling PBM from hemodialyzed patients and normal controls with OKT3, OKT4, and OKT8 monoclonal antibodies. When data were expressed as the OKT4/OKT8 ratio (Fig. 1), no consistent

abnormalities in the relative proportions of the main regulatory T-cell subsets was noted in hemodialyzed patients. In all patients the test was performed twice at 1-month intervals with minimal variations in the results obtained. However, when the absolute counts of each separate subset were considered, one observed significantly lower values in patients as compared to control subjects (Table 1), reflecting the lymphocytopenia present in these patients (the mean \pm SEM of lymphocytes per mm³ in patients was 1364 ± 476 and in controls was 1950 ± 430).

Lymphokine Activities. The biological activities of lymphokines IL-2 and the BCGFs were evaluated in parallel in the supernatants from stimulated E-rosetting T-cell cultures. Semipurified E-rosetting T cells were used instead of total mononuclear cells to eliminate the bias caused by the variable number of monocytes that usually contaminate lymphocyte suspensions from hemodialyzed patients. Here again, the test was performed twice, at 1-month intervals, with good consistency in the results.

IL-2 activity. In 9 of the 12 patients studied, the IL-2 activity was either undetectable or close to background levels (Table 2). Only 3 patients showed significant IL-2 levels: 1 of them (MAG) presented a normal IL-2 activity, whereas the other 2 (JMA and SAM) exhibited depressed IL-2 values as compared to normal controls, although notably higher than the activity of the other patients.

To exclude the possibility that the observed defect was due to an abnormal refractoriness of patients' lymphocytes to the mitogenic stimulus, in seven cases, lymphocytes were cultured with supramitogenic concentrations of PHA (4 μ g/ml). Although IL-2 activity increased when using higher concentrations of the mitogen, it always remained lower in hemodialyzed patients when compared to control subjects (Table 3). It also was verified that no excessive prostaglandin E₂ production by remnant monocytes present in the culture was inhibiting IL-2 synthesis [monocytes can depress IL-2 production by releasing prostaglandin E₂, which in turn activates specific suppressor T lymphocytes (15)]. Prostaglandin E₂ levels in culture supernatants were measured by means of a biological assay (16). Prostaglandin E₂ levels in patients' supernatants were found to be low in the normal range [patients (mean \pm SEM), 2.42 ± 0.76 μ g/ml; normal controls (mean \pm SEM), 3.59 ± 0.36].

B cell-proliferating activities (BCGFs). B lymphocyte-enriched cellular suspensions from healthy donors were incubated with anti-IgM (μ chain) antibody in the presence of culture supernatants from mitogen-activated lymphocytes of patients or normal donors. Most patients showed normal B cell-proliferating activity. This normal BCGF activity contrasted with the abnormally low IL-2 values detected in the same culture supernatants (Table 1).

Sensitivity of Peripheral T Cells to Exogenous Recombinant IL-2. The presence of IL-2 receptors at the surface of patients' lymphocytes was tested by analyzing their capacity to proliferate in the presence of exogenous recombinant IL-2. As already shown by others, purified T lymphocytes from normal controls only expressed the IL-2 receptor when preactivated by mitogen or antigen stimulation and, thus, subsequently proliferated in the presence of IL-2 (5). At variance with this normal behavior, resting peripheral E-rosetting T cells from hemodialyzed patients showed a

significant capacity to proliferate in the presence of exogenous IL-2 independently of any prior stimulation (Fig. 2). These results were corroborated by immunofluorescence labeling with the anti-Tac monoclonal antibody, which specifically reacts with the human IL-2 receptor. As shown in Fig. 2, the proportion of Tac⁺ cells was significantly higher in hemodialyzed patients than in normal controls. The T-cell nature of these Tac⁺ cells has been confirmed by double-labeling experiments. First, the monoclonal antibodies UCHT1 (murine IgG1) and anti-Tac (murine IgG2a) were used in combination, thus showing that the Tac⁺ lymphocytes were also UCHT1⁺ (data not shown). Second, the 3B8 monoclonal antibody, which specifically recognizes human LGL, also has been tested in combination with the UCHT1 antibody (11). In fact, a particular subset of lymphocytes has been described bearing both T-cell and LGL markers, which are able to proliferate in the presence of IL-2 (17). This particular cell subset expresses lymphocyte-activated killer (LAK) activity (17, 18). In the hemodialyzed patients that were analyzed, cells expressing the 3B8⁺ UCHT1⁺ phenotype were not detected among the E-rosetting population (data not shown). All these double-labeling experiments were performed on the E⁺ cells of patients both before and after *in vitro* culture with recombinant IL-2.

Finally, the IL-2 receptor expression after PHA stimulation was comparable in hemodialyzed patients and normal controls (data not shown).

Sensitivity of Peripheral B Cells to BCGF I, BCGF II, and Recombinant IL-2. Peripheral B lymphocytes from hemodialyzed patients presented a normal response to exogenous lymphokines (Fig. 3). BCGF I and recombinant IL-2 induced a moderate and dose-related proliferation of resting B cells and did costimulate with anti-IgM (μ chain) antibody. The stimulatory effect mediated by BCGF II was strictly dependent on the presence of anti-IgM (μ chain) antibody, both in patients and controls (Fig. 3).

DISCUSSION

The aim of the present study was to analyze in parallel different phenotypic and functional immune parameters in patients undergoing chronic hemodialysis. As already reported by others (19, 20), the relative proportions of helper/inducer (OKT4⁺) and cytotoxic/suppressor (OKT8⁺) T cells were found to be normal in these patients. However, in accordance with previous reports (15, 16) and in line with the lymphocytopenia commonly observed in chronic uremia, significantly lower absolute counts of both regulatory lymphocyte subsets were observed as compared to normal controls.

In parallel, we analyzed the production by patients' lymphocytes of two soluble mediators playing a central role in T- and B-cell cooperation—namely, IL-2 and the BCGFs—and the response of the T and B cells of patients to these lymphokines.

Human IL-2, formerly termed T-cell growth factor, is a glycosylated protein (15–18 kDa) produced by lymphocytes after mitogenic or antigenic stimulation (6, 21). IL-2 has been purified to homogeneity, and its gene has been cloned, making the protein available as recombinant material (22). Originally, IL-2 was thought to act exclusively on T cells, but recent data have shown that it could also affect B cells (23, 24). Other lymphokines distinct from IL-2 are present in the supernatants of stimulated peripheral blood lymphocytes that induce B-cell proliferation (7, 8). The first BCGF that has been identified—namely, BCGF I—has a molecular mass of 14–18 kDa (25, 26). Its T-cell origin has been confirmed by reports describing the establishment of a human T-cell hybrid producing this lymphokine (27, 28). Subsequently, an additional BCGF activity termed BCGF II was isolated from the

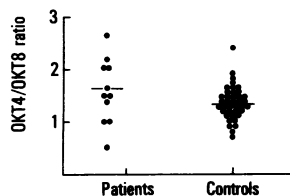


FIG. 1. Peripheral regulatory T-cell subset distribution in hemodialyzed patients. Results are expressed by the OKT4/OKT8 ratio (mean \pm SEM, 1.63 ± 0.19 in patients and 1.61 ± 0.09 in normal controls).

Table 1. Peripheral T-cell subsets in hemodialyzed patients

	OKT3 ⁺ cells		OKT4 ⁺ cells		OKT8 ⁺ cells	
	%	Cells per mm ³	%	Cells per mm ³	%	Cells per mm ³
Patients (n = 11)	57.09 ± 3.25	763 ± 268*	33.73 ± 2.23	450 ± 158 [†]	22.82 ± 2.46	305 ± 158 [‡]
Normal controls (n = 27)	68.44 ± 1.68	1334 ± 290	41.16 ± 1.49	803 ± 202	26.84 ± 1.21	523 ± 176

**P* < 0.0001 as compared to normal control subjects.

[†]*P* < 0.001 as compared to normal control subjects.

[‡]*P* < 0.01 as compared to normal control subjects.

supernatants of stimulated human lymphocytes and had a molecular mass of ≈50 kDa (29, 30). From a functional viewpoint, both lymphokines induce the proliferation of anti-IgM (μ chain) antibody-stimulated normal purified B cells (14). However, in contrast to BCGF I, BCGF II is unable to elicit a proliferative response on normal resting B cells [in the absence of anti-IgM (μ chain) stimulation] (14). IL-2 as well as the BCGFs are only active on target cells that express specific receptors for the lymphokine. The lymphokine-receptor interaction triggers cell function and modulates the receptor half-life at the cell surface. Receptors for IL-2 and the BCGFs are exclusively expressed after appropriate specific or nonspecific stimulation and, thus, can be considered as activation markers. Monoclonal antibodies have been produced in mice against human IL-2 and BCGF I receptors (ref. 9, S. Suzuki, personal communication).

The data reported in this study reveal an important distinction between the activities of IL-2 and the BCGFs detected in the supernatants of mitogen-stimulated cell culture from hemodialyzed patients. BCGF activity was found to be normally produced by nearly all of the lymphocytes from patients tested, thus contrasting with the significantly decreased IL-2 activity (close to background levels) found in 80% of patients.

This difference was associated with the distinct reactivity of the lymphocytes from patients to the two lymphokines. B lymphocytes from patients, like normal B cells, were able to proliferate in the presence of partially purified BCGF I, BCGF II, or recombinant IL-2, provided an appropriate first-activation signal [e.g., anti-IgM (μ chain) antibody] was given. No proliferative response was induced on the B cells

Table 2. Lymphokine activities detected in stimulated T-cell culture supernatants

Patient	B cell-proliferating activity*		IL-2 activity, units per ml
	1:10	1:20	
TUR	4.6	1.3	0.10
MON	—	—	0.20
HAD	8.6	2.8	0.11
HAM	7.8	2.0	0.14
AND	8.6	2.7	0.10
CHA	10.7	2.3	0.10
DAM	6.8	1.5	0.10
CHI	9.5	1.4	0.10
JMA	12.3	1.8	0.68
MAG	11.5	2.8	1.10
SAM	—	—	0.48
ABO	13.2	4	0.20
Mean ± SEM	9.33 ± 0.82 [†]	2.26 ± 0.26 [†]	0.28 ± 0.09 [‡]
Normal mean ± SEM	12.01 ± 0.73	2.91 ± 0.62	1.02 ± 0.16

*Results with 1:10 and 1:20 dilutions are expressed by the proliferation index defined in *Patients and Methods*.

[†]Mean values are not significantly different from the levels of normal controls.

[‡]*P* < 0.001 as compared to normal controls.

of patients or controls by BCGF II in the absence of anti-IgM antibody stimulation.

Moreover, BCGF I and recombinant IL-2 elicited the same pattern of response on resting B cells from controls and patients. At variance with this normal B-cell behavior, an abnormal response of resting T cells from hemodialyzed patients to exogenous recombinant IL-2 was observed. In fact, whereas IL-2 only induced the proliferation of normal purified T lymphocytes that had previously been activated by an antigen or a mitogen, the resting T lymphocytes from patients were highly responsive to exogenous recombinant IL-2. Taken together with the increased proportion of patient T cells recognized by the anti-Tac monoclonal antibody that selectively binds to the IL-2 receptor, these data indicate the existence of circulating preactivated T cells in hemodialyzed patients. The IL-2 binding sites present on T cells from patients are probably high-affinity receptors, since a proliferative response is elicited by IL-2 on these cells [high- and low-affinity receptors for IL-2 have been described (31); both of them are recognized by the anti-Tac monoclonal antibody, but only the former mediate proliferation]. The increased T-cell IL-2 receptor expression may account, at least in part, for the decreased IL-2 activity detected in patients' lymphocyte supernatants by provoking increased absorption of the lymphokine. At this point it is important to underline that no evidence has been found in the hemodialyzed patients studied so far for the presence of increased proportions of peripheral LGL cells expressing the T3 marker and able to respond to IL-2. This particular cell subset has been shown to mediate a nonspecific cytolytic activity (lymphocyte-activated killer activity) (17, 18). In fact, no doubly-labeled cells were found among the E⁺ cell populations from patients analyzed (both before or after *in vitro* culture in presence of exogenous IL-2) when using an anti-T3 monoclonal antibody (UCHT1) in combination with the 3B8 antibody that specifically recognizes human LGL cells (16).

It is interesting that Raska *et al.* (16) in their series of hemodialyzed patients did not find any increase in the proportions of circulating T cells expressing HLA-DR antigens (usually present on activated T cells). This discrepancy with our own findings underlines the interest of studying several activation markers in parallel. It will be important to compare the IL-2 receptor and HLA-DR antigen expression in the same patients and, if the discrepancy is confirmed, to investigate its significance.

Table 3. IL-2 activity induced by stimulation with two different mitogen concentrations

	IL-2 activity, units per ml	
	PHA at 0.4 μg/ml	PHA at 4 μg/ml
Hemodialyzed patients (n = 7)	0.35 ± 0.06*	0.95 ± 0.12 [†]
Normal controls (n = 9)	0.91 ± 0.14	3.44 ± 0.35

*Mean ± SEM; *P* < 0.01 as compared to normal control subjects.

[†]Mean ± SEM; *P* < 0.001 as compared to normal control subjects.

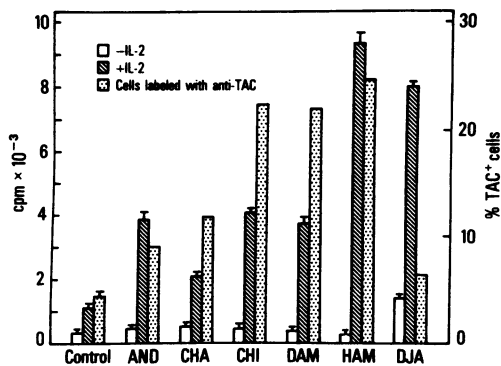


FIG. 2. Sensitivity of freshly purified resting T cells from hemodialyzed patients (AND, CHA, CHI, DAM, HAM, and DJA) and normal controls, to exogenous recombinant IL-2. In parallel, the proportions of Tac⁺ cells (in the same T-cell populations) are reported.

The mechanisms of T-cell preactivation observed in hemodialyzed patients are uncertain. It is well known that the physical contact of circulating cells with dialysis membranes may stimulate the release of several mediators, notably interleukin 1 by monocytes (32). However, the reason why the abnormal preactivation observed selectively involves T lymphocytes without affecting B cells remains an open question. Moreover, although interleukin 1 plays an important role in the regulation of IL-2 production, it does not induce the expression of IL-2 receptors in the absence of an additional activation signal. One may also hypothesize that the blood transfusions (administered as part of pretransplantation protocols or because of anemia) could activate T cells by inducing in these patients the production of circulating anti-T cell autoantibodies, distinct from anti-HLA antibodies (unpublished data), or by provoking latent viral infections (hepatitis B virus, Epstein-Barr virus, human immunodeficiency virus . . .).

The existence of preactivated T cells could have several important physiopathological consequences. For example, the increased IL-2 receptor expression may lead to its release in the circulation. Rubin *et al.* (33) have recently shown that the IL-2 receptor may indeed be found in soluble and active

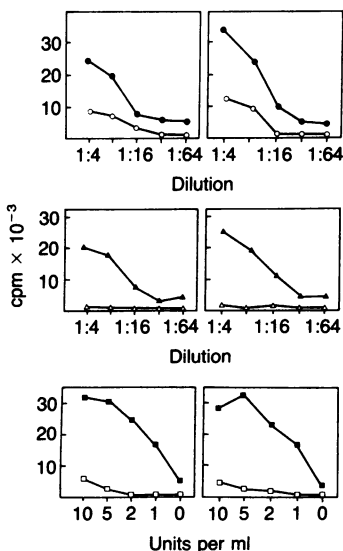


FIG. 3. Sensitivity of B lymphocytes from a hemodialyzed patient (Left) and a control (Right) to exogenous purified BCGF II (Top), BCGF II (Middle), and recombinant IL-2 (Bottom). The data in this figure are from one representative experiment; all of the patients analyzed showed the same B-cell reactivity pattern. ○, △, and □, cultures performed in the absence of anti-IgM (μ chain) stimulation; ●, ▲, and ■, cultures performed in the presence of anti-IgM (μ chain) stimulation.

form in culture supernatants of stimulated T cells as well as in human sera. It will be of interest to know if hemodialyzed patients show an increased IL-2 receptor release that could interfere with regulatory processes. It also would be important to determine the functions and putative deleterious actions of the described preactivated cells. They could act by generating aberrant immune or even autoimmune responses or, alternatively, by suppressing immunity, providing then an explanation to the depressed cell-mediated reactivity observed in hemodialysis patients.

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