Clin. exp. Immunol. (1992) **87**, 493–498

IL-2 infusion abrogates humoral immune responses in humans

D. J. GOTTLIEB, H. G. PRENTICE, H. E. HESLOP, C. BELLO & M. K. BRENNER
Department of Haematology, Royal Free Hospital, London, England

(Accepted for publication 16 September 1991)

SUMMARY

Although IL-2 infusion enhances cell-mediated cytotoxicity in patients with neoplastic disease, administration is paradoxically associated with a modest fall in total serum IgG and an increased risk of infection. We now show that the adverse effects of IL-2 infusion on the humoral immune system are substantial. Although IL-2 induces the B cell growth and differentiating factors IL-4 and IL-6, infusion abrogates primary antibody responses entirely and reduces secondary antibody responses 50-fold following antigen challenge. There is no evidence of the generation of cells with suppressive activity on B cells but IL-2 increases the ratio of circulating virgin:memory cells. These results may help to explain the increased rate of bacterial infection in patients receiving IL-2. As IL-2 plays a central role in the generation of an immune response, the finding that it is also sufficiently immunosuppressive to inhibit primary- and secondary-type antibody responses suggests that exploration of the underlying mechanisms may provide insights into immune system homeostasis and may offer new approaches to therapeutic immunosuppression.

Keywords IL-2 B cells immunoglobulin humoral responses

INTRODUCTION

Infusion of IL-2 has marked effects on cellular immune function, generating activated killer cells which can induce tumour regression in patients with malignant disease [1,2]. By contrast, the effects of IL-2 infusion on humoral responses have not been characterized. *In vitro*, IL-2 may increase B cell growth and differentiation either directly or by induction of B cell stimulatory cytokines [3,4]. *In vivo*, the combination of local injection of low-dose IL-2 with a vaccine antigen usefully augments the subsequent antibody response in initially non-responding subjects [5]. However, there are indications that high-dose IL-2 infusion may actually diminish humoral immunity, since total serum IgG levels fall and nosocomial infection rates rise in patients so treated [6–8]. To determine whether the fall in IgG and rise in infection rate were manifestations of significant suppression of humoral immunity, we immunized patients pre-IL-2 infusion with a priming antigen (keyhole limpet haemocyanin (KLH)) and measured subsequent production of specific IgM and IgG antibodies. We also analysed the effect of IL-2 on the less readily suppressible secondary antibody response, by immunizing the patients with the recall vaccine tetanus toxoid (TT).

Correspondence: Dr D. J. Gottlieb, Department of Haematology, Westmead Hospital, Hawkesbury Rd, Westmead, NSW 2145, Australia.

PATIENTS AND METHODS

Patients

Eighteen patients (nine with acute myeloid leukaemia, five with acute lymphoblastic leukaemia, and four with multiple myeloma) were studied in the recovery period following chemotherapy or autologous bone marrow transplantation (ABMT). Eleven patients (eight men, three women) in the control group had a median age of 36 years (range 17–58). They were vaccinated with antigen but received no post-vaccination immunotherapy. Seven patients (four men, three women) with a median age of 45 years (range 20–55) were immunized with antigen prior to receiving infusions of IL-2 as described below. All patients had received courses of myelo-ablative chemotherapy (Table 1).

ABMT was performed after conditioning with cyclophosphamide 120 mg/kg body weight and total body irradiation 750 cGy in a single fraction for patients with acute leukaemia or after melphalan 200 mg/m^2 intravenously alone prior to autologous marrow re-infusion for patients with multiple myeloma. Patients received IL-2 according to a clinical study of immunotherapy in minimal residual haematological malignancy [9]. Patients with acute lymphoblastic leukaemia were excluded because of the risk of disease acceleration. Patients refusing consent or presenting during a period when IL-2 was not available were used as controls. Patients with acute lymphoblastic leukaemia receiving myeloablative induction or consolidation therapy were included in the control group. Their serological responses to vaccination were studied and found to

Table 1. Characteristics of patients vaccinated with KLH or tetanus toxoid prior to immunotherapy with IL-2 and controls not receiving post-vaccination immunotherapy

	Control	IL-2
n	11	7
Age (years)		
Range	17–58	20–55
Median	36	45
Sex (M/F)	8/3	4/3
Diagnosis		
AML	4	5
MM	2	2
ALL	5	
Treatment		
	7 ABMT 4 Chemotherapy (1 mito/araC 1 DAT10 2 UKALLX)	3 ABMT 4 Chemotherapy (1 mito/araC 1 DAT10 2 HiDoAraC)

AML, acute myeloid leukaemia; MM, multiple myeloma; ALL, acute lymphoblastic leukaemia; ABMT, autologous bone marrow transplant. Mito/araC, mitozantrone 12 mg/m^2 i.v. D1-5, cytosine arabinoside 100 mg/m^2 i.v. D1-5; DAT10, daunorubicin 45 mg/m^2 i.v. D1,3,5, cytosine arabinoside 100 mg/m^2 i.v. D1-10, thioguanine 100 mg/m^2 o D1-10; HiDoAraC, cytosine arabinoside 1-2 g/m^2 i.v. b.d. D1-5; UKALLX, daunorubicin 45 mg/m^2 i.v. D1,2, etaposide 100 mg/m^2 i.v. D1-5, cytosine arabinoside 100 mg/m^2 i.v. D1-5, vincristine 1.5 mg/m^2 i.v. D1, prednisone 40 mg/m^2 o D1-5.

be identical to those of patients treated for acute myeloid leukaemia (data not shown).

Vaccination

Patients were vaccinated 1–3 weeks after either chemotherapy or ABMT. To assess recall responses, deep s.c. vaccination with TT 40 U adsorbed onto aluminium hydroxide (Wellcome, Beckenham, UK) was used. All patients had prior exposure to Tetanus toxoid, a minimum of one year before study. Responses to primary antigen exposure were assessed after vaccination with the neoantigen KLH. KLH was purchased as an ammonium sulphate study from Cambridge Biosciences (Cambridge, UK) and purified as previously described [10]. For vaccination, 200 μl of purified KLH protein were injected into the deep s.c. tissue of the upper arm.

IL-2 infusion

Full clinical details of IL-2 treatment have been published elsewhere [9]. Patients in this study received recombinant IL-2 cloned in *Escherichia coli* (Glaxo, Geneva, Switzerland) by continuous i.v. infusion at doses from 160 to 600 μg/m^2 per day ($0{\cdot}5$–$1{\cdot}2 \times 10^6$ U/m^2 per day). Patients receiving IL-2 were vaccinated with TT and/or KLH immediately before the commencement of infusion, which was started 1–3 weeks after chemotherapy or ABMT when circulating neutrophil counts had exceeded $0{\cdot}5 \times 10^9$/*l*. Control patients were vaccinated with TT and/or KLH at the same time following chemotherapy/ABMT, but did not receive IL-2 infusion.

Serum immunoglobulin levels during IL-2

Serum immunoglobulins were measured on an Encore centrifugal analyser using an immunoturbidometric method. Serum was diluted with a semi-automatic Hook and Tucker diluter. A commercially purchased standard (human serum protein calibrator; Dakopatts, Glostrup, Denmark) was used as a reference material to construct a standard curve from which test values were calculated.

ELISA for specific antibodies to injected antigens

Patient sera were collected immediately prior to vaccination, weekly for 1 month, fortnightly for a second month and monthly thereafter. Serum antibody responses to injected antigens were determined by analysis in an ELISA. Flat-bottomed 96-well microtitre plates (Nunc, Roskilde, Denmark) were coated overnight with either TT 5 U/ml, 50 μl/well (Wellcome) or KLH 1 mg/ml, 100 μl/well (Cambridge Biosciences). After washing, plates were blocked for 2 h to prevent non-specific binding, washed again and serial dilutions of test specimens added. Plates were incubated at 37°C for 2 h and, after further washing, an alkaline-phosphatase-conjugated anti-IgG or IgM (Sigma, St Louis, MO) added. The degree of binding was measured using *p*-nitrophenyl phosphate substrate (Sigma) which was added to all wells. Optical density was read at 405 nm on a Titertek Multiscan MC ELISA plate reader. Test values were determined by comparison with a reference serum as previously described [10,11].

Immunophenotyping

Mononuclear cells were immunophenotyped using a two-colour indirect immunofluorescence technique in a microtitre plate system [12]; 1–2×10^5 cells washed with PBS with 0·2% azide before staining with RFB-7 antibody (to the CD20 antigen), a kind gift of Professor G. Janossy; and CD45R and UCHL1 antibodies to isoforms of the leucocyte common antigen, a kind gift of Professor P.C. Beverley. After incubation and washing, cells were stained with a rhodamine-conjugated goat anti-mouse IgM or IgG antibody (Southern Biotechnology, Birmingham, AL). After washing, 200 cells were examined using Nikon AFX-II fluorescent microscope.

IL-4 and IL-6

IL-4 was measured by a two-site immunoradiometric assay using two MoAbs. Wells of a flat-bottomed microtitre plate were coated with the first anti-IL-4 antibody (10 μg/ml), blocked and serial dilutions of test samples added. After incubation and washing a second ^{125}I-labelled anti-IL-4 antibody was added. Excess label was washed off and wells were counted in Compugamma gamma counter. Samples were standardized against an IL-4 standard from the National Institute of Biological Standards and Controls (Hertfordshire, UK) (89/509). The assay has a detection limit of 30 pg/ml of IL-4.

IL-6 was measured with a bioassay technique [13]. An IL-6-dependent cell line, B9 (B13.29) was used and the assay calibrated with an interim IL-6 standard from the National Institute for Biological Standards and Controls (88/51, 6250 units/1.25 μg per ampoule).

Detection of cytokine mRNA in cells during IL-2 infusion

Cytoplasmic RNA was extracted from peripheral blood mononuclear cells (PBM) using the detergent lysis method [14].

Briefly, cells were washed in PBS and resuspended in hypotonic buffer (10 nM Tris–HCl, pH 7·8, 150 mM NaCl) containing 10 mM Vanadylribonuclease complexes (BRL, Uxbridge, UK) and 1% nonidet P40 (Shell Chemicals, UK). The nuclei were pelleted by centrifugation and the cytoplasmic extract mixed with an equal volume of extraction buffer (40 mM Tris–HCl, pH 7·8, 40 mM EDTA, pH 8·0, 700 mM NaCl) and 20% SDS. The supernatant was extracted twice with phenol/chloroform and once with chloroform/isoamyl alcohol. RNA was precipitated after adding 2·5 volumes 100% ethanol, and stored at −70°C. Northern blotting was performed after RNA was dissolved in 7 μl of TE, added to an equal amount of denaturing buffer (10% MOPS, 10% formaldehyde, 80% formamide) and heated at 65°C for 15 min. RNA (20–50 μg) was loaded into 1% denaturing gels containing 6% formaldehyde and electrophoresed at 100 mV for 4 h. RNA was transferred to nitrocellulose filters which were baked, pre-hybridized (in 50% formamide, 5 × SSC, 5 × Denhardt's solution and 50 μg/ml denatured salmon sperm DNA) and hybridized with ^{32}P-labelled IL-4 or IL-6 probe for 16 h at 42°C. Filters were washed and exposed to Fuji film for 1–10 days at −70°C.

RESULTS

Cytokine mRNA and serum levels during IL-2 infusion

We studied the expression of mRNA for IL-4 and IL-6, two cytokines important in B cell growth and differentiation, before and during IL-2 infusion. Transcripts for both cytokines became detectable in peripheral blood lymphocytes (PBL) during IL-2 infusion (Fig. 1) and the corresponding proteins are secreted by cells cultured *ex vivo* (data not shown). IL-6 levels in serum increased during IL-2 infusions (mean pre-IL-2, 22 ± 19 pg/ml *versus* 213 ± 116 pg/ml on IL-2), but despite the appearance of IL-4 mRNA in PBL no increase in serum levels was seen.

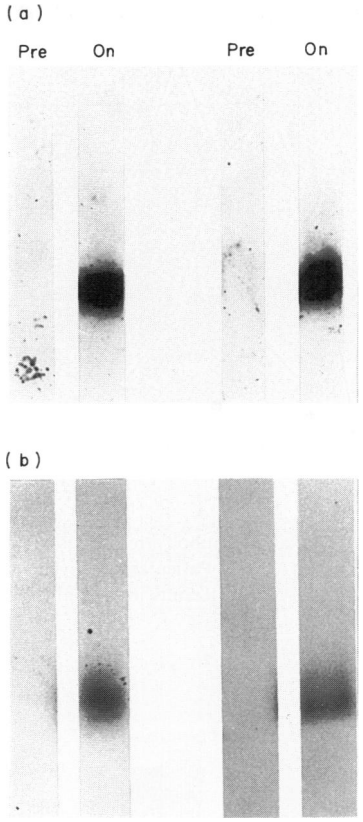

Fig. 1. Northern blot analysis of total cellular RNA obtained from patients before and during IL-2 infusion. Peripheral blood mononuclear cells were isolated on density gradients and RNA extracted using the detergent lysis method. RNA was electrophoresed on agar gel and transferred to nitrocellulose filters. Filters were hybridized to ^{32}P-labelled cDNA probes to IL-4 (a) and IL-6 (b) fragments. Equal loading of gels was established using a probe to the constitutively expressed cellular protein tubulin. Autoradiographs from the hybridized nitrocellulose filters are illustrated.

Serological responses to vaccination with priming and re-call antigens induced by IL-2

Patients were immunized with a priming antigen KLH immediately before IL-2 infusion was started. In approximately 70% of normal donors, this antigen induces a two-fold or greater rise in IgM response within 14 days and a four-fold rise in IgG antibody within 21 days [15]. Five out of nine patients not receiving IL-2 produced an IgM response greater than two-fold and seven out of nine developed an IgG response greater than four-fold. In contrast, none of six patients receiving IL-2 mounted any detectable IgM or IgG response even when followed up to 8 weeks beyond immunization and 6 weeks beyond their last exposure to IL-2. These results are illustrated in Fig. 2 (a and b), showing the patterns of response in four patients, two receiving immunization with KLH alone and two receiving KLH followed by IL-2.

Secondary or recall antibody responses are substantially harder to suppress than are responses to a neo-antigen. To assess the extent of IL-2-induced humoral immune suppression we therefore immunized IL-2 recipients and control patients with TT. Approximately 90% of normal donors produce a greater than four-fold rise in IgG antibodies within 2 weeks of re-exposure [16], as did nine out of 11 patients vaccinated after chemotherapy/ABMT and not receiving IL-2. In contrast, only one out of six of the patients receiving IL-2 produced such a rise and this response was of abnormally brief duration (< 4 weeks). These results are illustrated in Fig. 2c.

Data from all patients studied are summarized in Fig. 3, which shows the area under the antibody curve (antibody titre × duration) for the different groups. The IgG anti-KLH responses of vaccinated patients not receiving IL-2 were not significantly different from those of normal donors, while IgM antibody was present at a lower level than in normals ($P = 0{\cdot}02$). However, anti-KLH antibody responses of both IgM and IgG isotype were entirely abrogated in patients receiving IL-2 infusion ($P < 0{\cdot}001$). For TT, there was no significant difference in the level of response between normal donors and patients receiving antigen alone, but the response of patients receiving antigen followed by intravenous IL-2 was reduced approximately 50-fold.

Serum immunoglobulin levels in patients receiving IL-2

One explanation for the apparent failure of specific antibody responses to injected antigens might be extravascular redistribution of immunoglobulin caused by the leaky capillary syndrome which accompanies IL-2 administration [17]. To examine this, we measured serum immunoglobulin levels in patients before, during and 48–72 h after IL-2 infusion (Table 2). Mean levels of

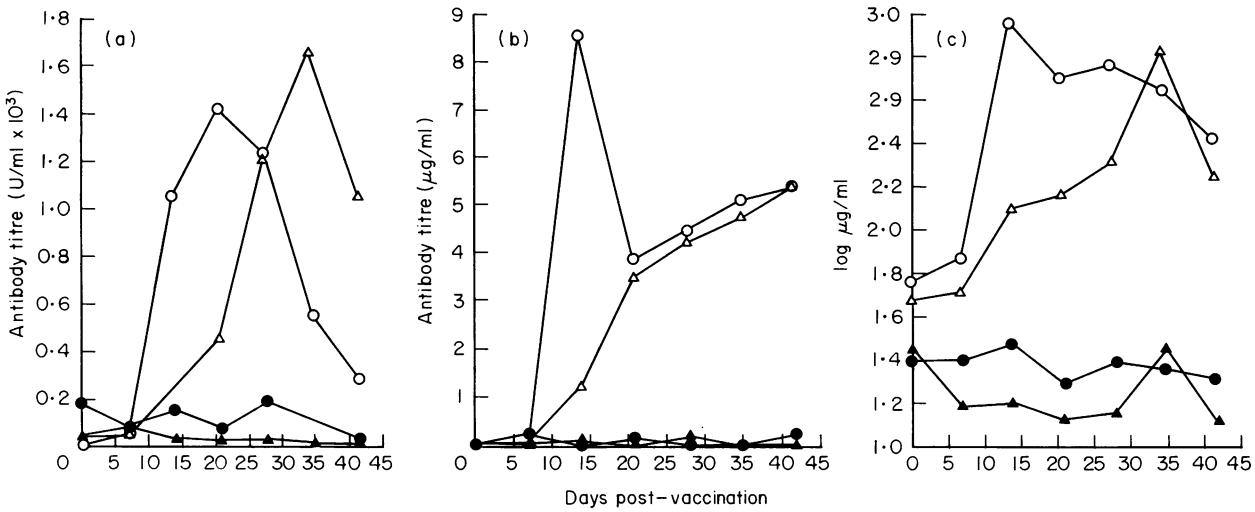

Fig. 2. Representative antibody responses after vaccination with KLH (a, IgM; b, IgG) or TT (IgG, c) for patients following chemotherapy or ABMT. Patients were given vaccine alone (○, chemotherapy; △, ABMT) or vaccine and IL-2 (●, chemotherapy + IL-2; ▲, ABMT + IL-2). Results in (a) and (b) are from the same patients.

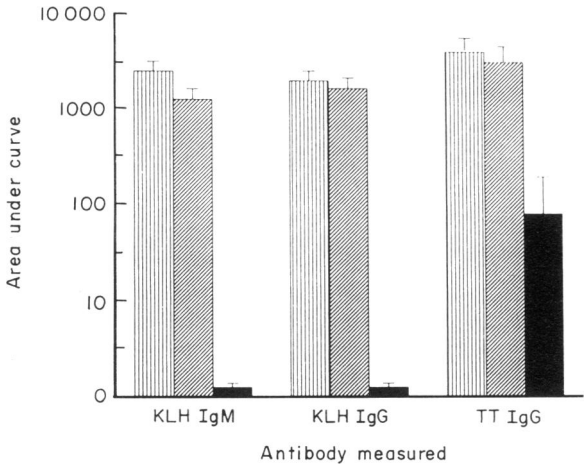

Fig. 3. Mean area under the curve for antibody responses to KLH and TT in normal donors (▩), patients receiving antigen alone (▨) and patients receiving antigen and IL-2 infusion (■). Unpaired *t*-testing of antibody titres obtained from all groups shows no significant difference between normal donors and patients receiving antigen alone for KLH IgG and TT IgG, but a highly significant effect for IL-2 infusion ($P < 0.0001$ for KLH IgM and IgG and for TT IgG).

Table 2. Serum immunoglobulin levels (±s.e.m.) immediately before, during and 48–72 h after IL-2 infusion. Normal values are shown in parentheses

	Before	During	After
IgG (8–18 g/*l*)	10·7±0·9	8·4±0·8†	9·3±1·3*
IgA (0·9–4·5 g/*l*)	1·6±0·3	1·2±0·3†	1·6±0·4
IgM (0·6–2·8 g/*l*)	0·8±0·1	0·5±0·1	0·8±0·1

Significantly different from pre-IL-2 value: *$P < 0.05$, †$P < 0.001$.

all immunoglobulin isotypes are in the low normal range pre-infusion. Falls in IgG and IgA levels during IL-2 infusion were modest, returning towards normal within 48 h of stopping IL-2. Within 1 week, immunoglobulin levels for all three isotypes had returned to normal. Moreover, delay in the re-entry of immunoglobulin into the vascular space following cessation of IL-2 did not account for impaired responses, since specific antibody could not be detected even in patients followed for up to 8 weeks after initial vaccination and 7 weeks after the completion of IL-2.

B lymphocyte number and function during IL-2 infusion

Since IL-2 induces cells with the ability to lyse autologous lymphocytes [18], lysis of B cells by IL-2-activated lymphokine-activated killer (LAK) cells could explain absent antibody responses. Total mononuclear cell numbers fell from $1{\cdot}3 \pm 0{\cdot}4 \times 10^9/l$ pre-infusion to $0{\cdot}8 \pm 0{\cdot}2 \times 10^9/l$ during IL-2, and the percentage of cells expressing the CD20 antigen fell simultaneously from 4·7±3·8% to 3·3±2·2% (P not significant). The fall in B cell numbers was therefore modest and reflected a general reduction in the number of lymphocytes expressing all phenotypes, rather than a specific fall in B cell numbers during IL-2 infusion [19].

To examine the function of circulating B cells, Ficoll-separated mononuclear cells from patients were cultured in the presence of the T cell-dependent B cell stimulator pokeweed mitogen (PWM) for 5 days and the supernatants were examined for the presence of total IgG and IgM by ELISA. *In vitro* secretion of IgG fell during IL-2 infusion, and IgM secretion rose in comparison with pre-IL-2 levels (Table 3). However, these changes were not statistically significant. We depleted $CD8^+$, $CD16^+$ or both subsets of cells prior to culture with PWM to determine whether IL-2-activated T or natural killer (NK) cells might be responsible for reducing antibody responses *in vivo*. Increased immunoglobulin secretion from subset depleted PBM was no greater during IL-2 than pre-infusion (Table 3), suggesting that IL-2 does not inhibit antibody responses by augmenting the function of inhibitory T or NK cells.

Table 3. Immunoglobulin secretion *in vitro* by PWM-stimulated peripheral blood mononuclear cells before and during IL-2 infusion

	Before IL-2 (μg/ml)	During IL-2 (μg/ml)	% Increase after depletion of		
			CD8	CD16	CD8/16
IgG	19±7	12±2	144 (133)	98 (95)	177 (203)
IgM	19±9	36±14	172 (150)	126 (93)	213 (441)

PBM were cultured in RPMI/10% FCS with PWM 10 μg/ml. Supernatants were harvested after 5 days and assayed for total IgG and IgM by ELISA. The effect of depletion of lymphocyte subsets by MoAb and complement is shown. Figures indicate the percentage increase in immunoglobulin secretion during IL-2 infusion by lymphocyte depleted populations in comparison to the complement treated control. For comparison, the figures in parentheses give the same measurements taken pre-IL-2. Results are the mean of five separate experiments. Antibodies for depletion: for CD8, RFT8, kind gift of Professor G. Janossy, Department of Immunology, Royal Free Hospital; for CD16, Leu 11b Becton-Dickinson, Mountain View, CA. Two exposures to a 1:2·5 dilution of baby rabbit complement (Buxted Rabbit, UK) were used for complement lysis. In all experiments, residual cells post-depletion were < 5% total population.

Table 4. Percentage of circulating mononuclear cells positive for CD45A and CD45O (UCHL1) antigens before and during IL-2 infusion

IL-2 course no.	$CD45A^+$		$UCHL1^+$	
	Before IL-2	During IL-2	Before IL-2	During IL-2
1	16	18	73	66
2	36	35	60	29
3	43	50	44	34
4	35	31	49	40
5	20	19	50	30

Data show results from five courses of IL-2. IL-2 evoked a significant reduction in the percentage of cells expressing UCHL1 ($P = 0·03$) but no significant difference in expression of CD45A. Similar results were found within the $CD8^+$ lymphocyte subpopulation.

Effect of IL-2 on lymphocyte expression of leucocyte common antigen (LCA)

Different isotypes of the LCA component designated CD45A and CD45O (UCHL1) distinguish virgin (unprimed) $CD45RA^+UCHL1^+$ from memory (primed) $CD45A^-UCHL1^+$ lymphocytes. Memory cells are responsible for the majority of immune reactivity including T cell help for B cell growth and differentiation [20]. *In vitro*, treatment of lymphocyte populations with IL-2 induces a reduction in the proportion of cells expressing the virgin phenotype with a concomitant increase in the proportion of cells expressing the primed phenotype [2]. In contrast, when the distribution of the LCA components on circulating PBM was studied during IL-2 infusion, no change in the percentage of cells expressing the CD45RA antigen was found (Table 4). However, a consistent fall in the percentage of cells expressing the primed $CD45A^-UCHL1^+$ phenotype was observed (Table 4).

DISCUSSION

IL-2 administration to patients in remission of haematological malignancy produces immune system activation, inducing the production of LAK cells and the release from PBL of the cytokines tumour necrosis factor (TNF) and interferon-gamma (IFN-γ) [9,21]. Here we show that during IL-2 infusions mRNA for the cytokines IL-4 and IL-6 becomes detectable in PBL. When lymphocytes from patients receiving IL-2 are cultured *ex vivo*, the corresponding proteins also appear in cell supernatants and IL-6 levels in the serum increase. We speculated that these changes would enhance B cell function and augment the responses to vaccine antigens since IL-4 and IL-6 are B cell growth and differentiation factors and since both TNF and IFN-γ which are released during IL-2 infusion increase B cell proliferation and antibody synthesis [22,23]. Moreover, IL-2 itself can act as a T cell replacing factor in humans [24], and a proportion of B cells are IL-2-receptor-positive and proliferate on exposure to the cytokine [25].

Paradoxically, however, the systemic administration of high-dose IL-2 infusions has entirely different consequences for humoral immunity. Serological responses to priming and recall antigens are suppressed. As both secondary (recall) and primary antibody responses were affected, the degree of immunosuppression is substantial. The results cannot be attributed to extensive redistribution of serum immunoglobulin, since falls in total immunoglobulin are modest and no responses were detected even when patients were followed for at least 8 weeks after antigen exposure and the completion of IL-2 infusion. Nor are the effects mediated by IL-2-dependent induction of inhibitory cytotoxic cells [26], since removal of CD8 (cytotoxic T) and/or CD16 (NK/LAK) lymphocytes from cultured patient B cells restores neither immunoglobulin secretion nor *in vitro* responsiveness to mitogen which is impaired during IL-2 infusion (data not shown).

Impairment may instead be at the level of T helper–B cell interaction. IL-2 infusion is associated with an increase in the ratio of naive (unprimed) to memory (primed) lymphocytes. Accumulation of cells expressing the $CD45RA^+$ phenotype, which are relatively non-immunoreactive, may be responsible for impairing the T cell help available to B cells. Recent evidence suggests that lymphokines including IL-6 regulate CD45A expression as part of a homeostatic immune mechanism [27]. The induction of IL-4 may also induce a down-regulatory signal, since IL-4 counteracts the helper effect of IL-2 on antigen-activated B cells *in vitro* if added during the first 48 h of culture [28]. Under normal circumstances, IL-4 and IL-6 present at high concentrations at sites of antigen challenge would induce proliferation and differentiation in pre-activated B cells and simultaneously limit the immune response. However, i.v. administration of IL-2 may generate physiological homoeostatic mechanisms on a systemic scale and thus abort specific antibody responses before they become established. In support of this hypothesis is the observation that inhibition of B cell responses represents part of a wider spectrum of immune suppression induced by IL-2 since impairment of cell-mediated immunity and neutrophil function during IL-2 infusions have also been described [6,29].

Whatever the mechansm by which IL-2 inhibits these processes, the loss of responsiveness to antigenic challenge probably contributes to the increased risk of bacterial infection

now documented in patients receiving IL-2 infusion [7,8]. Patients receiving IL-2, particularly if given after conventional chemoradiotherapy, clearly require assiduous clinical attention and a low threshold for the use of i.v. antibiotics. Antibiotic prophylaxis has recently been reported to reduce the incidence of catheter-related sepsis in patients receiving IL-2 [30] and should be considered given the difficulties in infection surveillance these patients pose. Further analysis of the underlying mechanisms should provide insights into the physiology of immune regulation and indicate methods for reversing IL-2-induced immune suppression. Such knowledge may also indirectly suggest therapeutic strategies for the treatment of diseases in which aberrant antibody production plays a pathogenic role.

REFERENCES

1 Rosenberg SA, Lotz MT, Muul LM, *et al.* A progress report on the treatment of 157 patients with advanced cancer using lymphokine-activated killer cells and interleukin-2 or high-dose interleukin-2 alone. N Engl J Med 1987; **316**:889–97.

2 Gottlieb DJ, Prentice HG, Heslop HE, Bello-Fernandez C, Bianchi ACM, Galazka AR, Brenner MK. Effects of recombinant interleukin 2 administration on cytotoxic effector function following intensive chemo-radiotherapy for hematological malignancy. Blood 1989; **74**:2335–42.

3 Howard M, Matis L, Malek TR, *et al.* Interleukin 2 induces antigen-reactive T cell lines to secrete BCGF-1. J Exp Med 1983; **158**: 2024–39.

4 Ralph P, Jeong G, Welte K, *et al.* Stimulation of immunoglobulin secretion in human B lymphocytes as a direct effect of high concentrations of IL2. J Immunol 1984; **133**:2442–5.

5 Meuer SC, Dumann H, Meyer zum Buschenfelde KH, Kohler H. Low-dose interleukin-2 induces systemic immune responses against HBsAg in immunodeficient non-responders to hepatitis B vaccination. Lancet 1989; **i**:15–18.

6 Weibke EA, Rosenberg SA, Lotze MT. Acute immunologic effects of interleukin-2 therapy in cancer patients: decreased delayed type hypersensitivity response and decreased proliferative response to soluble antigens. J Clin Oncol 1988; **6**:1440–9.

7 Ades EW, Bosse D, Orr S, Gillespie T. In vivo and in vitro evaluation of immune function of patients receiving either IL-2 or IL-2 and LAK: rapid induction of anergy as a result of proliferation of cells with immune dysfunction. Abstract 725, Proc ASCO, March 1989.

8 Ramming K, Richards JM, Gilewski TA, Vogelzang NJ. Interleukin-2 (IL-2) induced risk for bacteremia. Abstract 763a, Proc ASCO, March 1989.

9 Gottlieb DJ, Brenner MK, Heslop HE, *et al.* A phase 1 clinical trial of recombinant interleukin 2 following high dose chemo-radiotherapy for haematological malignancy: applicability to the elimination of minimal residual disease. Br J Cancer 1989; **60**:610–15.

10 Wimperis JZ, Brenner MK, Prentice HG, Reittie JE, Karayiannis P, Griffiths PD, Hoffbrand AV. Transfer of a functioning humoral immune system in transplantation of T-lymphocyte-depleted bone marrow. Lancet 1986; **i**:339–43.

11 Wimperis JZ, Gottlieb DJ, Duncombe AS, Heslop HE, Prentice HG, Brenner MK. Requirements for the adoptive transfer of primary antibody responses in man. J Immunol 1990; **144**:541–7.

12 Campana D, Janossy G. Leukemia diagnosis and testing of complement-fixing antibodies for bone marrow purging in acute lymphoid leukemia. Blood 1986; **68**:1264–71.

13 Aarden LA, De Groot ER, Schapp OL, Lansdorp PM. Production of hybridoma growth factor by human monocytes. Eur J Immunol 1987; **17**:1411–16.

14 Maniatis T, Fritsch EF, Sambrook, J. Molecular cloning: a laboratory manual. Cold Spring Harbour: Cold Spring Harbour Laboratory Press, 1982.

15 Amlot PL, Hayes AE, Gray D, Gordon-Smith EC, Humphrey JH. Human immune responses in vivo to protein (KLH) and polysaccharide (DNP-Ficoll) neoantigens: normal subjects compared with bone marrow transplant patients on cyclosporine. Clin Exp Immunol 1986; **64**:125–35.

16 Saxon A, Stevens RH, Ashman RF. Regulation of immunoglobulin production in human peripheral blood leucocytes–cellular interactions. J Immunol 1977; **118**:1872–9.

17 Cotran RS, Pober JS, Gimbrone MA Jr, *et al.* Endothelial activation during interleukin 2 immunotherapy. A possible mechanism for the vascular leak syndrome. J Immunol 1987; **139**:1883–8.

18 Oshimi K, Oshimi Y, Saito H, Mizoguchi H. Cytotoxicity of interleukin-2 activated lymphocytes for autologous normal blood mononuclear cells J Immunol Methods 1988; **109**:161–8.

19 Smith LJ, Brown MH, Towe D, Callard RE, Beverley PCL. Functional subsets of human helper-inducer cells defined by a new monoclonal antibody, UCHL1. Immunology 1986; **58**:63–70.

20 Akbar AN, Amlot PL, Timms A, Lombardi G, Lechler R, Janossy G. The development of primed/memory $CD8^+$ lymphocytes in vitro and in rejecting kidneys after transplantation. Clin Exp Immunol 1990; **81**:225–31.

21 Heslop HE, Gottlieb DJ, Bianchi ACM, *et al.* In vivo induction of interferon-γ and tumour necrosis factor by interleukin 2 infusion following intensive chemotherapy or autologous marrow transplantation. Blood 1989; **74**:1374–80.

22 Romagnani S, Giudizi MG, Biagotti, *et al.* B cell growth factor activity of interferon-gamma. Recombinant interferon-gamma promotes proliferation of anti-IgM activated human B lymphocytes. J Immunol 1986; **136**:3513–16.

23 Jelinek DF, Lipsky PE. Enhancement of human B cell proliferation and differentiation by tumor necrosis factor-alpha and interleukin 1. J Immunol 1987; **139**:2970–6.

24 Callard RE, Smith SH, Shields JG, Levinsky RJ. T cell help in human antigen-specific antibody responses can be replaced by interleukin 2. Eur J Immunol 1986; **16**:1037–42.

25 Waldmann TA, Goldman CK, Robb RJ, *et al.* Expression of Interleukin 2 receptors on activated human B cells. J Exp Med 1984; **160**:1450.

26 Suzuki H, Yamashita N, Maruyama M, Yoshikawa T, Yano S. Human lymphokine-activated killer cells suppress pokeweek mitogen-induced immunoglobulin synthesis. Clin Exp Immunol 1989; **78**:406–11.

27 Brod SA, Rudd CE, Purvee M, Hafler DA. Lymphokine regulation of CD45R expression on human T cell clones. J Exp Med 1989; **170**:2147–52.

28 Llorente L, Crevon MC, Karray S, Defrance T, Banchereau J, Galanaud P. Interleukin (IL) 4 counteracts the helper effect of IL2 on antigen-activated human B cells. Eur J Immunol 1989; **19**:765–9.

29 Klempner MS, Noring R, Mier JW, Atkins MB. An acquired chemotactic defect in neutrophils from patients receiving interleukin-2 immunotherapy. N Engl J Med 1990; **322**:957–65.

30 Bock SN, Lee RE, Fisher B, *et al.* A prospective randomised trial of evaluating prophylactic antibiotics to prevent triple-lumen catheter-related sepsis in patients treated with immunotherapy. J Clin Oncol 1990; **8**:161–9.