Immunology 1991 **72** 584–587

ADONIS 001928059100099K
BRIEF COMMUNICATION

Enhancement of a delayed hypersensitivity reaction to a contact allergen, by the systemic administration of interleukin-2

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Accepted for publication 28 December 1990

SUMMARY

The immunopharmacological effects of interleukin-2 (IL-2) on the sensitization and effector phases of the delayed-type hypersensitivity (DTH) reaction were studied using contact sensitivity to the haptenizing agent dinitrochlorobenzene (DNCB). When administered at the time of priming to DNCB, IL-2 had no effect on the subsequent magnitude of the response. Interleukin-2 was, however, able to increase the magnitude of the response when given at the time of secondary challenge; the degree of change was directly related to the dose of IL-2. The proportions of T cells in the draining lymph node and spleen of IL-2-treated animals decreased by approximately one-third, but there was no alteration to the balance between $CD4^+$ and $CD8^+$ T cells. The results suggest that the increase in DTH observed was due to a pharmacological effect rather than to an increase in T-cell number.

The cytokine interleukin-2 (IL-2) has been the focus of many studies aimed at understanding and manipulating the immune response, particularly against malignant diseases, since its description in 1976.[1] The provision of this material in large amounts was made possible by the expression of the gene in bacterial cells,[2] and its use for the treatment of human malignant disease was quickly investigated.[3]

The role of IL-2 in the clonal expansion and differentiation of T cells[4] led also to investigation of its potential for the therapy of immunodeficiency diseases. Initial studies *in vitro* showed that IL-2 could reverse the diminished immune response seen in aged animals[5] or those treated with agents such as cyclosporin A.[6] In humans, the suppressed immune responses associated with leprosy[7] and rheumatoid arthritis[8] have also been replenished with IL-2. Human *in vivo* studies have concentrated on cancer patients, but some work has been carried out to test the usefulness of IL-2 for the treatment of microbial infections, such as leprosy and leishmaniasis.[9] With the exception of toxicity, the major effect of IL-2 in humans is the change in levels of circulating lymphocytes, with an early, severe depletion being followed by a significant increase in the numbers of lymphoid cells in the peripheral blood, on continued administration of IL-2.[10]

As part of an investigation into the immunopharmacological properties of cytokines, we investigated the influence of high-dose IL-2 on the priming and effector phases of a T-cell mediated response in a normal, healthy animal. The status of the cell-mediated immune response was measured as the delayed-type hypersensitivity (DTH) reaction to 1-chloro-2,4-dinitrobenzene (DNCB). The sensitizing properties of DNCB are related to its ability to haptenize epidermal proteins, particularly collagen, by forming covalent bonds with the ε-amino group of lysine residues, and an antibody response does not normally arise.

Mice (10–12-week-old, BALB/c females) were primed by painting the shaven neck with 50 μl of a 10% (w/v) solution of DNCB in diethyl ether (Sigma, St Louis, MO); this was taken as Day 0. Mice were challenged on the footpad at Day 6 with a 1% solution and the DTH response taken as footpad swelling after 24 hr. Recombinant human IL-2 was reconstituted to 15,000 U/ml in sterile water and subsequently diluted (as required) in sterile phosphate-buffered saline (PBS). The IL-2 was from Glaxo (Geneva, Switzerland); its specific activity was described as being $1{\cdot}6 \times 10^6$ U/mg (CTLL-2-3 assay) with an endotoxin contamination of $< 7{\cdot}0$ U/mg (limulus assay) and it was considered suitable for administration to humans. Each injection of 15 kU represented a dose of 750 kU/kg (20 g mice, average). Sham-treated mice received phosphate-buffered saline (PBS) alone.

Mice (in groups of 10–15) received six intraperitoneal, 1 ml injections of IL-2 or PBS at 8-hr intervals (i.e. over 2 days), according to the following schedule: protocol 1 (15 kU IL-2 or PBS only), with the first injection at or before the time of priming (Day or Day-2) *or* at or before the time of secondary challenge (Day 6 or Day 5); protocol 2 (dose response of IL-2), first injection 24 h prior to challenge (Day 5). The results are

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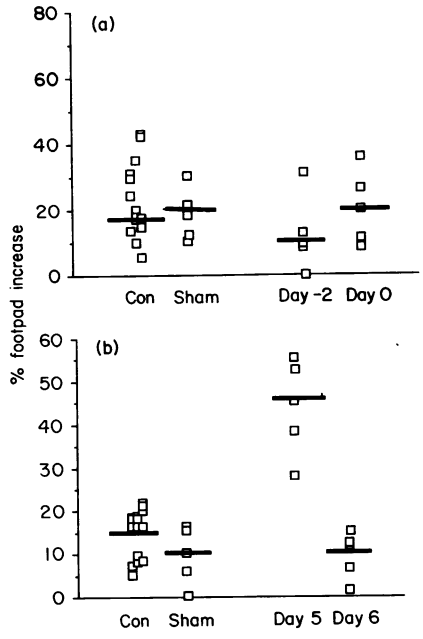

Figure 1. The DTH response to DNCB following exposure to high-dose (15 kU) IL-2 at the priming or effector phases. The percentage increase in footpad swelling is shown for each test animal, with the mean increase for the group indicated by a bar. A scattergram has been used to indicate the degree of variability within each group, particularly the sham-treatment group. (a) Animals were exposed to 15 kU human IL-2 at the priming stage of the response. (b) Animals were exposed to 15 kU human IL-2 at the effector phase of the response.

expressed as the percentage increase in swelling between the test footpad and the sham-treated (ether alone) contralateral footpad of individual animals, with statistical analysis by the Mann–Whitney *U*-test. The phenotype of lymphoid cells in the spleens and popliteal lymph nodes (PLN) draining the test footpads was determined by cytofluorographic analysis on an EPICS Profile.1 (Coulter Electronics, Luton, Beds, U.K.), using phycoerythrin-linked monoclonal antibodies directed against L3T4 (CD4), Ly-2 (CD8) Ly-5 (a B-cell marker) and MAC (CD11b; used here to indicate the presence of monocytes), obtained from Coulter Electronics.

Typical results from experiments conducted under protocol 1 are shown in Fig. 1. In neither case did the response of the sham-treated (PBS alone) group vary from that of the control. Figure 1 shows the effect of pretreating with IL-2 48 hr prior to priming and/or beginning IL-2 at the same time as priming. It will be seen that animals receiving 15 kU IL-2 at these times did not subsequently develop a DTH response different from the control groups, and so we concluded that IL-2 could not alter the priming response. In Figure 1b, it can be seen that those treated with 15 kU IL-2 beginning 24 hr prior to secondary challenge (Day 5) exhibited a greatly enhanced ($P < 0{\cdot}0005$) swelling, but mice given IL-2 from the time of secondary challenge (beginning Day 6) were once again no different from the control.

When the phenotypes of spleen and lymph node cells from control, sham and 15 kU IL-2-treated animals were compared (Table 1), it was clear that two distinct effects were occurring. In the draining lymph node and with the exception of a decrease in the proportion of MAC$^+$ cells (30–15%), there was no difference between the control and sham-treated groups. Administration of IL-2 caused a decrease in the proportion of T cells in the PLN by approximately one-third, with CD4$^+$ and CD8$^+$ cells being equally affected. There was a corresponding increase (36–53%) in the percentage of Ly-5$^+$ cells (B cells) and MAC$^+$ cells (15–34%) present in the popliteal lymph node (PLN) of IL-2-treated mice. The effects on the splenic lymphoid populations was less clear, because the sham-treated group was very different from the control (all cell types reduced). Since these animals were subjected to any stress associated with the therapy, they represent the correct group with which to compare the IL-2-treated group. Under these circumstances, there was a large rise in the proportion of B cells and macrophages present, with no real effect on T-cell populations, although the CD4:CD8 balance was slightly affected. When the weights of the spleens were examined, it was found that the IL-2-treated group had the heaviest spleens ($P < 0{\cdot}00001$), while the sham group was no different from the untreated animals (IL-2, 172 ± 24 mg; sham, 106 ± 22 mg; untreated, 131 ± 15 mg).

Table 1. Effect of high-dose (15 KU) IL-2 on lymphocyte populations in the spleen and draining lymph node at the effector phase of the DTH response

	Treatment group		
Cell type	Control	Sham	IL-2
Lymph node			
L3T4$^+$	36·8*	39·5	23·5
Ly-2$^+$	12·6	12·8	8·4
Ly-5$^+$	31·8	35·8	52·9
MAC$^+$	30·0	14·9	33·7
Spleen			
L3T4$^+$	60·0	33·7	29·0
Ly-2$^+$	11·5	4·9	6·2
Ly-5$^+$	25·3	17·0	38·1
MAC$^+$	34·9	25·0	42·2

* Results are expressed as the percentage of cells in the total population, following cytofluorographic analysis.

Because there was a significant increase in DTH when IL-2 was given at Day 5, we then investigated the dose–response nature of this increase. Typical results from this experiment (protocol 2) are shown in Fig. 2. An appreciable increase in swelling was apparent with the administration of 5 kU IL-2, which became significant ($P < 0{\cdot}005$) over the control at 7·5 kU.

The results presented here show clearly that IL-2 has profound effects on the effector phase of a DTH response to the contact allergen DNCB, but no effect on the initial sensitisation. These results concur with earlier findings relating to the ability of IL-2 to augment secondary, but not primary responses.[11]

The recognized ability of IL-2 to stimulate division of T cells *in vivo*[12] led us to investigate whether T cells could be 'primed' with IL-2 to be more responsive to antigen. We also wished to know whether IL-2 could influence T-cell activity once antigen had entered the system. It was for this reason that we investigated the effect of pretreating animals with IL-2 prior to

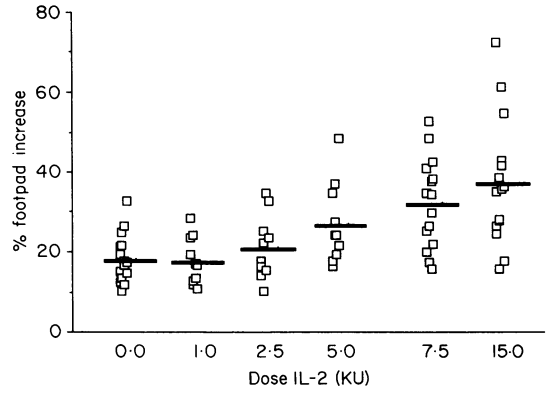

Figure 2. Dose–response effect of IL-2 on the effector phase of a DTH response. Mice were sensitized to DNCB and IL-2 was administered at the doses shown, on Day 5. Twenty-four hours after IL-2 treatment was started, the mice were challenged with DNCB and the degree of swelling noted after a further 24 h. The data are presented as described for Fig. 1.

initial (Day −2) and secondary (Day 5) exposure to DNCB as well as on the days of priming (Day 0) and challenge (Day 6). The only time that IL-2 was effective was when given prior to secondary challenge.

The augmentation of the DTH response by IL-2 may be explained in part by the different functions associated with the so-called 'T-helper-1' and 'T-helper-2' cells in mice. IL-2 secretion is associated with T-helper-1 cells[13] and these cells themselves are the recognized mediators of hypersensitivity in a range of systems[14]. Inflammatory effector cells (such as macrophages) can express IL-2 receptors[15] and could therefore be open to hyperstimulation by high levels of IL-2. Further evidence for this comes from the fact that the effect of IL-2 on the DTH response was linear with the dose and was not subject to a 'threshold effect', such as might be expected if additional effector cells were being activated. In addition, the well-recognized effects of IL-2 on vascular endothelium[12] would contribute to this effect by increasing the ease of cellular migration into the site of antigenic challenge.

This effect was accompanied by changes in the lymphoid populations in the associated lymph node, but changes in the splenic populations were partly obscured by the effects of stress associated with the procedure. The effect of stress on the immune system cannot therefore be under-estimated or forgotten. A profound loss of T cells from the PLN was observed in the 15 kU IL-2 group, but this is probably related to the mobilization of leucocytes observed in IL-2 treatment of human subjects[10] and the recognized ability of IL-2 to increase the migratory capability of stimulated T cells,[16] rather than to an IL-2-induced increase in the numbers of B cells and macrophages. The increased splenic weight in the IL-2-treated group is wholly consistent with previous observations of IL-2-induced leucocyte proliferation in tissues.[12] While IL-2 administration can lead to the *in vivo* generation of LAK-type cells, there was no evidence for the presence of a large unstained population within the spleens of IL-2-treated mice, which would be expected from the expansion of cells from the NK lineage, as LAK cells are. While it is possible that IL-2 was inducing preferential expansion of B cells and monocytes over T cells in this organ, we believe that the low proportion of T cells in this group, in the context of an increased splenic size, was due to a combination of proliferation[12] and migration[16] on the lymphocyte populations examined.

Thus, it is clear that IL-2 can act as a pharmacological agent to enhance a T-cell mediated response (delayed hypersensitivity), suggesting that the untreated response is suboptimal. Such suboptimal responses can occur if antigen-primed T cells are exposed to antigen in the absence of antigen-presenting cells[17] and this can be reversed by IL-2 *in vitro*.[18] Similarly, the aquisition of neonatal tolerance can be reversed by systemic administration of IL-2.[19,20] The results reported here may reflect these phenomena. If, when primed T cells re-encounter haptenized collagen at the challenge site it was only correctly presented to a proportion, then the remainder may become anergic. Thus, a balance would be struck between these cells and those which responded. The presence of IL-2 would alter this balance by reducing the proportion of anergic cells and the magnitude of the response would increase. While it is reasonable to expect that such immunomodulatory effects of IL-2 might be primarily exerted on T cells themselves, the fact that the effect was not seen on the priming part of the response when initial clonal expansion takes place (Day 0), nor as an effect on re-activated T cells following secondary challenge (Day 6), suggests an alternative (or interacting) mechanism on non-T-cell components of the response. In particular, IL-2 begun on Day 5 (before secondary challenge) may have compromised the integrity of the endothelial tissue[12] such that non-T cells could much more readily gain access to the area of secondary challenge, with a resultant increase in swelling. Careful work will be required to define the contribution of these two mechanisms to the overall response.

Although exposure to high levels can have profound effects on a variety of cell types *in vitro*, the pharmacokinetics of IL-2 *in vivo* demonstrate that it is cleared very quickly, with human recombinant IL-2 having a serum half-life of under 2 min[21] in mice. The effect on cells would therefore be one of regular (in this case, eight-hourly) pulsing with high dose IL-2 and not of continuous exposure. This may go some way to explaining why IL-2 has not proven to be a particularly efficacious therapy for cancer, despite the clear presence of activated, specific T cells within the tumour.[22] Clearly, the potential role of IL-2 as a therapeutic agent remains to be fully realized, but our results suggest that it may be useful in conditions where hypersensitivity responses are recognized as playing an important part in the curative process, such as leishmaniasis and leprosy.[23]

ACKNOWLEDGMENTS

This work was supported in part by the Cancer Research Campaign (L.P.Walsh). The authors are indebted to Glaxo for the gift of pharmacological grade human IL-2. Y. Zaloom is supported by a scholarship from the World Association of Muslim Youth.

REFERENCES

1. Morgan D.A., Ruscetti F.W. & Gallo R. (1976) Selective *in vitro* growth of human T lymphocytes from bone marrow. *Science*, **193**, 1007.
2. Rosenberg S.A., Grimm E.A., McGrogan M., Doyle M., Kawascli E., Koths K. & Mark G.F. (1984) Biological activity of recombinant human interleukin-2 produced in *E. coli*. *Science*, **223**, 1412.

3. MULE J.J., SHU S. & ROSENBERG S.A. (1985) The anti-tumour activity of lymphokine-activated killer cells and recombinant interleukin-2 *in vivo*. *J. Immunol.* **135,** 646.
4. KERN D.E., GILLIS S., OKADA M. & HENNEY C.S. (1981) The role of interleukin-2 (IL-2) in the differentiation of cytotoxic T-cells: the effect of monoclonal anti-IL-2 antibody and absorption with IL-2 dependent T-cell lines. *J. Immunol.* **127,** 1323.
5. THOMAN M.L. & WEIGLE W.O. (1982) Cell mediated immunity in aged mice: an underlying lesion in IL-2 synthesis. *J. Immunol.* **128,** 2358.
6. WANG B.S., HEACOCK E.H., ZHENG C., TILNEY N.L. & MANNICK J.A. (1982) Restoration of allogeneic responsiveness of lymphocytes from cyclosporin-A treated animals with interleukin-2. *Transplantation*, **33,** 454.
7. HAREGEWOIN A., GODAL T., MUSTAFA A., BELEHU A. & YEMANEBERHAN T. (1983) T-cell conditioned media reverse T-cell unresponsiveness in lepromatous leprosy. *Nature*, **303,** 342.
8. EMERY P., PANAYI G.S. & NOURI A.M.E. (1984) Interleukin-2 reverses deficient cell mediated immune responses in rheumatoid arthritis. *Clin. exp. Immunol.* **57,** 123.
9. KAPLAN G., KEISSING R., TEKLEMARIAM S., HANCOCK G., SHEFTEL G., JOB C.K. *et al.* (1989) The reconstitution of cell-mediated immunity in cutaneous lesions of lepromatous leprosy by rIL-2. *J. exp. Med.* **169,** 893.
10. LOTZE M.T., MATORY Y.L., ETTINGHAUSEN S.E., RAYNER A.A., SHARROW S.O., SEIPP C.A.Y., CUSTER M.C. & ROSENBERG S.A. (1985) *In vivo* administration of purified human interleukin-2, II. Half-life, immunologic effects and expansion of peripheral lymphoid cells in vivo with recombinant IL-2. *J. Immunol.* **135,** 2865.
11. ROSENBERG S.A., SPEISS P.J. & SCHWARZ S.L. (1983) *In vivo* administration of interleukin-2 enhances specific alloimmune responses. *Transplantation*, **35,** 631.
12. Ettinghausen S.E., Lipford E.H., Mule J.J. & Rosenberg S.A. (1985) Systemic administration of recombinant interleukin-2 stimulates in vivo lymphoid cell proliferation in tissues. *J. Immunol.* **135,** 1488.
13. CHERWINSKI H.M., SCHUMACHER J.H., BROWN K.D. & MOSMANN T.R. (1987) Two types of mouse helper T-cell clone, III. Further differences between Th1 and Th2 revealed by RNA hybridisation, functionally monospecific bioassays and monoclonal antibodies. *J. exp. Med.* **166,** 1229.
14. MOSMANN T.R. & COFFMAN R.L. (1989) Heterogeneity of cytokine secretion patterns and functions of helper T-cells. *Adv. Immunol.* **46,** 111.
15. MALKOVSKY M., LOVELAND B., NORTH M., ASHERSON G.L., GAO L., WARD P. & FIERS W. (1987) Recombinant IL-2 directly augments the cytotoxicity of human monocytes. *Nature*, **325,** 262.
16. Kornfield H., Berman J.S., Beer D.J. & Centre D.M. (1985) Induction of human T lymphocyte motility by interleukin-2. *J. Immunol.* **135,** 3887.
17. Lamb J.R., Scidmore B.J., Green N., Chiller J.M. & Feldmann M. (1983) Induction of tolerance in influenza virus immune T lymphocyte clones with synthetic peptides of influenza haemagglutinin. *J. exp. Med.* **157,** 1434.
18. ESSERY G., FELDMANN M. & LAMB J.R. (1988) Interleukin-2 can prevent and reverse antigen-induced unresponsiveness in cloned human T lymphocytes. *Immunology*, **64,** 413.
19. MALKOVSKY M., MEDAWAR P.B., THATCHER D.R., TOY J., HUNT R., RAYFIELD L.S. & DORE C. (1985) Acquired immunological tolerance of foreign cells is impaired by recombinant interleukin-2 or vitamin A acetate. *Proc. natl. Acad. Sci. U.S.A.* **82,** 536.
20. Morahan G., Allison J. & Miller J.F.A.P. (1989) Tolerance of class-I histocompatibility antigens expressed extrathymically. *Nature*, **339,** 622.
21. DONOHUE J.H., LOTZE M.T., ROBB R.J., ROSENSTEIN M., BRAZIEL R.M., JAFFE E.S. & ROSENBERG S.A. (1984) *In vivo* administration of purified human interleukin-2 in mice. *Cancer Res.* **44,** 1380.
22. LOTZE M.T. & FINN O.J. (1990) Recent advances in cellular immunology: implications for immunity to cancer. *Immunol. Today*, **11,** 190.
23. Turk J.L. & Bryceson A.D.M. (1971) Immunological phenomena in leprosy and related diseases. *Ad. Immunol.* **13,** 209.