ELSEVIER

Journal of Chromatography B, 662 (1994) 315–323

JOURNAL OF CHROMATOGRAPHY B: BIOMEDICAL APPLICATIONS

Review

Delivery of interleukin 2 for immunotherapy

David R. Kaplan

Department of Pathology, Case Western Reserve University, Biomedical Research Building, Room 926, 2109 Adelbert Road, Cleveland, OH 44106-4943, USA

Abstract

The local production of interleukin 2 (IL-2) by T lymphocytes acts to enhance the immune response by inducing growth and differentiation of a variety of immune cells. In clinical situations that require immunostimulation, such as vaccination and enhancement of tumor immunity, IL-2 therapy has been considered; however, the extraordinary toxicity of the drug inoculated systemically has greatly limited its application. Since the most serious toxic consequences of the drug are related to its systemic delivery, alternative strategies have been developed. Local delivery of the cytokine has been successfully used in some circumstances, and this form of delivery does not result in the life-threatening complications that limit systemic use. Liposome encapsulated IL-2 represents a mechanism to accentuate local delivery by causing a depot effect. Finally, the use of IL-2 has been predicated on the conception of the cytokine as an absolute monomer. Nevertheless, IL-2 spontaneously forms noncovalent and covalent self-associations. Because covalent dimers have been shown to initiate differential signalling in target cells, it is necessary to account for this property in devising and evaluating therapeutic protocols; moreover, it seems possible to use this property for modifying and regulating the therapeutic response.

Contents

1. Introduction

Recovery from infections and specific protection against reinfection depend upon the activity of lymphocytes. Both B and T lymphocytes possess clonally distributed receptors with each receptor specific for a single antigen from among the vast variety of foreign substances in our ecosystem. The lymphocytes reside in our bodies in an inactive state but when a lymphocyte is exposed to the specific antigen that binds to its receptor, that lymphocyte is specifically activated. Activation comprises 2 events: clonal expansion so that specific lymphocytes increase in frequency and differentiation so that mechanisms to effect immunity are manufactured by

0378-4347/94/$07.00
SSDI 0378-4347(94)00212-N

the cell. After activation cytokines are produced in abundance by a subset of T lymphocytes. It is the role of these cytokines to facilitate both clonal expansion and the differentiation of cellular functions.

Interleukin 2 (IL-2) is a cytokine produced in abundance by T lymphocytes after activation of the cells with antigen and it plays a major role in signalling cell growth and differentiation of a variety of different immune cells including T cells, B cells, macrophages, and natural killer cells [1]. Although there are other immune cytokines that play similar roles, the capacity of IL-2 in transducing signals in these cells is among the most powerful.

The capacity for IL-2 to induce proliferative signals in T lymphocytes is the property that accounts for the cytokine's discovery [2,3]. Conditioned supernatants from lectin-stimulated peripheral blood mononuclear cells were shown to enhance the proliferation of T cells in culture. Later the substance most responsible for this activity was defined as IL-2. The identity of IL-2 was solidified by cloning of the cDNA [4–6].

Once cDNA clones for IL-2 were available, a number of different expression systems were developed [7–13]. IL-2 was produced in large quantities in bacteria, yeast, insect cells, and eukaryotic cells. This capability allowed for a thorough investigation of the functional properties of the cytokine. Because IL-2 was produced as a purified homogeneous product, the activities seen could be clearly assigned to the molecule. The potent activity of IL-2 in inducing T cell proliferation was confirmed; furthermore, it was realized that the cytokine strongly promoted the growth of natural killer cells and markedly enhanced the level of cytotoxicity that both natural killer cells and T lymphocytes exhibited [14–19]. Since cytotoxicity was thought to play an important role in mediating immunity to various threats including neoplastic growth and viral infection, the possible use of IL-2 as a therapeutic tool was considered.

Cytokines in general and IL-2 in particular are not present in the systemic circulation. They are produced by a small number of cells in concentrations that allow for local activity. Consequently, the use of IL-2 for therapeutic purposes represented a new class of drug and the delivery of a cytokine drug was not obvious.

An apparent analog for cytokine drugs was hormones such as insulin. Hormones circulate systemically and are given intravenously as replacement therapy for their deficiency. It is reasonable that the establishment of cytokines for therapeutic purposes followed the lead of hormone therapy. The first use of cytokines involved systemic inoculation into animals in model systems and into patients for a number of different clinical indications.

In patients with certain solid tumors [20–23] and in animal models of cancer [24,25], IL-2 injected intravenously in large doses induced tumor regression. The cytokine is a potent antitumor agent and for some patients it has produced dramatic results. It is currently used in patients with renal cell carcinoma and some data with melanoma has also been encouraging. The mechanism of IL-2-mediated tumor regression has not been elucidated although it is likely that enhancement of both tumor-specific T lymphocytes and natural killer cell activity is involved in this effect.

IL-2 therapy has also been developed for IL-2 receptor positive leukemias in animal models [26]. In this case, a toxin has been conjugated to the IL-2 which specifically targets the toxin to the malignant cells, thereby directly suppressing tumor growth. The same conjugate has been proposed for use in inducing immunosuppression as desired in transplantation and autoimmunity [27–30]. IL-2 has the potential to specifically suppress the allogeneic immune response since inactive T cells do not express high affinity IL-2 receptors but activated T cells do.

Furthermore, IL-2 has been investigated in conjunction with vaccines to enhance the immunization especially in immunosuppressed individuals, and it has been used experimentally in the treatment of certain infectious diseases such as leprosy [31], disseminated cutaneous Leishmaniasis [32], infection with cytomegalovirus [33], septicemia with *Escherichia coli* [34], and disseminated *Mycobacterium avium* infection [35]. IL-2 has also been used in patients with

primary immunodeficiency syndromes [36,37] and human immunodeficiency virus infection [38,39]. Clinical improvement has been seen in these cases.

Although intravenous IL-2 therapy has been demonstrated to be useful, its major drawback has been the lack of targeting of the cytokine to its site of action. Cells throughout the body express receptors for the cytokine; consequently, intravenous IL-2 activates these cells indiscriminately. The cells at the tumor site or at the site of vaccination are not preferentially activated. To obtain effective local concentrations of IL-2 at the desired site of action, investigators have increased the doses of the cytokine injected; however, this approach has been limited by toxicity. Systemic activation of IL-2 receptor bearing cells results in extraordinary toxicity that limits the dose of cytokine that can be safely given [40–42]. IL-2 causes a vascular leak syndrome that can lead to severe hypotension and consequently systemic delivery of the cytokine can be life-threatening. Moreover, there are multiple other serious side effects of systemic levels of IL-2 that have also hampered the development of the cytokine for clinical uses.

IL-2 does not naturally circulate systemically. It is produced physiologically for local consumption. The toxicity of IL-2 seems to be a reflection of the imperfect analogy of hormone therapy with cytokine therapy. Recognition of the regional bias of IL-2 allows for the development of alternative strategies for therapeutic purposes.

2. Alternative delivery of IL-2

The toxicity of systemic delivery of IL-2 has prompted the development of alternative means of providing the cytokine. Perhaps one of the most exciting recent advances involves the use of gene transfer to provide for local production of IL-2. In these cases systemic levels of cytokine have not been reported and are not likely to be achieved. Instead production of IL-2 by targeted cells provides for cytokine at the specific site of action.

In an effort to produce vaccines that would better elicit protective immunity, investigators have engineered live viruses so that infection would result in the local production of IL-2. Recombinant vaccinia viruses have been produced that include IL-2 in an expression cassette so that cells infected with the virus produced IL-2 locally [43,44]. The recombinant vaccinia virus was more effective than the parental virus in acting as a vaccine in immunodeficient mice. Because the cytokine acts powerfully to promote the growth and differentiation of the cells of immunity, it is likely that the locally secreted IL-2 enhanced the immune response to the virus by stimulating the immune cells recruited to fight the vaccinating infection. This procedure for vaccination has been proposed for patients that are immunodeficient and consequently would benefit most from immunostimulation. This procedure would not be likely to produce systemic levels of cytokine commensurate with the levels achieved in patients given IL-2 for therapy of solid tumors. IL-2 dependent toxicity was not observed.

A second use of a vector directing the expression of IL-2 involves a murine model for solid tumors, cultured cell lines that produce tumors upon inoculation into naive mice. These cells have been transfected with vectors that encode for the expression of IL-2. They were then given to mice subcutaneously. The mice injected with unmodified cells produced tumors whereas the IL-2 producing tumor cells did not result in any solid tumors. More importantly, animals that received the IL-2 producing tumor cells were able to reject unaltered tumor cells subsequently injected [45]. This result has been interpreted to indicate that the local production of cytokine enhanced an immunologically specific response against the tumor cells. Again no IL-2 dependent toxicity was observed with this local modality of delivery.

The difficulty with the use of genetically altered cells to treat solid tumors in this way involves the requirement for the in vitro isolation of appropriate tumor cells, the procedure of transferring genes and selecting for transfectants, and the reinfusion of these genetically altered cells in a safe way. Since naturally occurring

tumors often comprise cells of different levels and vectors of differentiation, the feasibility of this procedure for patients might be questioned. Consequently, other investigators have pursued the local delivery of the cytokine itself.

The direct injection of IL-2 at the site of tumors provides a local concentration of the cytokine, and several investigators have demonstrated tumor regression after injection of IL-2 at the site of tumor growth [46–49]. Similarly, intradermal injection of recombinant IL-2 in patients with lepromatous leprosy has produced a significant systemic effect in decreasing the total body burden of *Mycobacterium leprae*, presumably by locally inducing immune cells that have the capacity to circulate. In these patients there was no drug toxicity observed [31].

Nevertheless, the potential of local IL-2 therapy for tumors or infections may be limited by the rapid diffusion of the cytokine away from the injection site. We have demonstrated that IL-2 attached to a solid phase produces a more powerful effect on the regression of tumors compared to the same amount of cytokine in soluble form [50]. Presumably the attachment of the cytokine to a plastic bead helped to retain the cytokine in the vicinity of the tumor and thereby a more potent anti-tumor effect was achieved.

IL-2 in liposomes has also been used to deliver the cytokine to a specific site. In one study the local delivery of liposome formulations of IL-2 gave significantly greater anti-tumor effects compared to empty liposomes or to soluble IL-2. Also in this study of metastatic lung lesions, local delivery was seen to be superior to systemic inoculation of the cytokine [51].

In another study investigators found that the inclusion of IL-2 in liposomes along with a variety of different immunogen such as the polysaccharide from *Pseuduomonas aeruginosa* [52], recombinant herpes simplex virus glycoprotein D [53], and the lipoprotein of Gram-negative bacterial cell wall [54] resulted in enhancement of the specific immune responses. In these cases the liposomes were given locally, in the peritoneal cavity, intranasally, or subcutaneously. These applications co-localize the delivery of the cytokine with the antigen in an analogous way to the recombinant vaccinia that produced IL-2 as well as a defined antigen.

Encapsulation of the cytokine produced a marked depot effect when the cytokine was given intrathoracically, subcutaneously, or intraperitoneally. The biodistribution and pharmacokinetics of the drug were greatly influenced in this study by both encapsulation and by route of administration [55].

In a comparative study of free IL-2 versus liposome encapsulated IL-2, investigators found that the liposome encapsulated IL-2 maintained higher serum levels when injected intravenously [56]. Nevertheless, serum levels are not necessarily the best predictor for biological responses for IL-2 since the cytokine acts locally to promote tumor regression or the stimulation of immune responsiveness.

Another method that co-localizes the cytokine with antigen for vaccination involves the production of a chimeric protein which includes both an IL-2 domain and an antigen domain. A fusion protein consisting of a fully functional IL-2 domain and an epitopically intact herpes simplex virus type 1 glycoprotein D domain has been produced [57]. Without any adjuvants this fusion protein elicited high titers of specific antibodies and strong cell-mediated immunity.

A similar approach has been developed for the immunotherapy of tumors. A fusion protein consisting of both IL-2 and an immunoglobulin chain specific for carcinoma cells has been produced [58]. The activity of the IL-2 and the immunoglobulin remained intact in the chimeric protein and consequently the in-vitro cytotoxic destruction of tumor cells expressing the relevant antigen was enhanced by localizing the cytokine to the tumor cells.

3. Activity measurements for modified IL-2

Alterations in the structure of a molecule accentuate the need to understand the activity of the molecule. Any modification of a molecule, such as IL-2, has the potential to alter its function. Consequently, the measurement of activity is an important aspect of developing

recombinant molecules for therapeutic purposes. This measurement is particularly significant for molecules that have been mutated or fused with other domains to produce totally new proteins.

IL-2 exemplifies this perspective. IL-2 bioactivity has been measured most often with an assay that involves the growth of T lymphocytes [59]. IL-2 receptor positive T cells are incubated with various dilutions of the cytokine in wells of a 96-well microtiter plate and after an overnight incubation thymidine incorporation is ascertained during a 4-h pulse. Although this assay does measure the relative amount of IL-2 by comparing the activity at the site of action, it does not assess all of the relevant pharmacodynamic properties that are important for the activity of the cytokine in a therapeutic protocol.

We found that a single amino acid change in the primary sequence of IL-2 could have profound effects measured in a biophysical assay based on the molecule's amphipathicity although there were no detectable differences in the classical IL-2 bioactivity assay [60]. IL-2 naturally synthesized by T cells partitioned completely into the aqueous phase in a solution of the detergent Triton X-114. Whereas the ser-125 mutated species partitioned completely into the detergent phase. Nevertheless the activity of these 2 forms is indistinguishable in the classical bioassay. Phase-separation analysis in Triton X-114 has been used as a discrete indicator of amphipathicity, and differences in this biophysical parameter of a molecule are likely to have important effects on pharmacodynamics.

This perspective is also illustrated with the studies of liposome encapsulated IL-2. Although the encapsulated IL-2 has been shown to possess superior pharmacokinetics and a greater effect on tumor regression than free IL-2, activity of these formulations in a classical IL-2 bioassay were identical [56].

Beyond the discrete changes in a molecule's structure or differences in formulation, posttranslational modification of a protein also has significant effects on its activity and needs to be considered carefully in designing a therapeutic protocol. A drug used for the treatment of a patient has a profile of pharmacodynamic properties that can greatly influence its activity at the site of action.

Since the recombinant molecule is poorly soluble at neutral pH and has a short half-life in serum, investigators have chemically modified IL-2 with an active ester of polyethylene glycol [61]. This modification did not alter the molecules capacity to induce T cell proliferation or to enhance cytotoxicity however, its serum half-life was greatly increased and its capacity to inhibit the growth of a sarcoma cell line in mice was likewise increased. Additional study also revealed that polyethylene glycol modified IL-2 reduced the immunogenicity of the cytokine, further enhancing its attractiveness as a therapeutic agent [62]. Characterization of the modified molecule was performed with the recognition of the multi-dimensional nature of pharmacological parameters. It has been compared with unmodified cytokine in mediating inhibition of tumor growth and has been shown to be superior in several studies, probably because of its more favorable pharmacokinetics [61,63].

Nevertheless, IL-2 chemically modified with polyethylene glycol possessed toxicity similar to parent compound [64]. In one study the issue of toxicity was addressed by locally injecting polyethylene glycol modified IL-2 directly into tumors instead of using an intravenous route of delivery. Besides causing tumor regression, the conjugated IL-2 induced the development of specific immunity against the tumor which would be transferred to naive animals by lymphocytes [65]. This finding is reminiscent of the results indicating that tumor cells genetically modified to express IL-2 induce a specific immune response; however, the ease of cytokine delivery is a significant advantage over tumor cell culture and gene transfer.

4. Biophysical properties of IL-2 that influence activity

Biophysical aspects of a molecule and the influence of these effects on biodistribution and pharmacodynamics have not thoroughly consid-

ered in regards to IL-2. The behavior of IL-2 in phase separation analysis exemplifies this perspective [60]. Another characteristic that has not been accounted for in designing therapeutic protocols is the marked propensity of IL-2 to bind to itself. Although IL-2 readily self-associates, this biophysical parameter of the molecule has not been thoroughly investigated in terms of its influence upon the activity of the cytokine nor upon its therapeutic use.

Noncovalent self-association of IL-2 has been demonstrated by a number of different methods [66–68]. Self-association has been observed in gel permeation chromatography and in fluorescence quenching which has shown that the K_d of self-association is 600 n*M* [67].

We have also found noncovalent IL-2 self-association in an unexpected circumstance [68]. Using standard procedures for covalent binding to either polystyrene or Sepharose beads, we prepared an immunoabsorbent with IL-2 attached. Nevertheless, we found that a majority of the IL-2 on the beads was attached noncovalently by self-association to the small proportion of the cytokine which had been covalently bound to the solid phase. Because the noncovalently bound self-associated IL-2 was spontaneously released from the solid surface under defined conditions, this form of the cytokine represents a slow-release formulation that simulates the secretion of IL-2 from an activated T lymphocyte. In a related series of experiments, we have shown that solid phase IL-2 has activity in vivo and that this form of the cytokine was considerably more active in a rat tumor model than an equivalent amount of soluble IL-2 [50]. Thus, IL-2 self-association is a property that may be exploited for the development of alternative means to deliver the drug. Moreover, the marked propensity for the cytokine to self-associate may influence the activity or the pharmacokinetics of the drug given intravenously.

In spite of the potential of self-association to influence the therapeutic usefulness of IL-2, there have been few accessible assays for this property. We have recently developed an ELISA that measures the binding of IL-2 to itself. Moreover, we have developed an alternative bioassay for IL-2 that can distinguish between self-associated IL-2 and monomeric cytokine [69]. The classical IL-2 bioassay does not distinguish among self-associated and unmodified cytokine. In the prolonged proliferation after pulse assay IL-2 receptor positive cells are pulsed with a source of IL-2 in serum-containing medium for 1 h at 4°C and then they are plated in microtiter wells and thymidine incorporation is assessed every day for 1 week. Our results indicate that concentrations of IL-2 in the self-associating range, greater than 400 n*M*, give more proliferation in this assay than concentrations that give negligible self-association, for instance 100 n*M*, in the prolonged proliferation after pulse assay. In the classical IL-2 bioassay these 2 concentrations are indistinguishable.

5. Covalently self-associated IL-2

Besides studies on noncovalent self-association of IL-2, we have detected IL-2 covalent multimers on SDS-PAGE and Western Blotting [60]. The spontaneously formed IL-2 dimers and trimers were not affected by reduction in 2-mercaptoethanol indicating that the covalent multimers were formed with crosslinks other than disulfide bonds. We suggested in that manuscript that transglutamination of the IL-2 was the likely explanation for our results. Furthermore, we found that isolated dimers spontaneously reformed monomers. It is our assumption that the covalent self-association of IL-2 is related to the noncovalent self-aggregating properties inasmuch as noncovalent interactions are a prerequisite for covalent bonding.

Another group of investigators has found a naturally occurring form of self-associated IL-2 produced by cells from fish regenerating nervous tissue, and they have recently shown that this form of the cytokine, in contrast to the monomeric form, is cytotoxic for oligodendrocytes. The naturally occurring dimeric IL-2 was covalently linked by transglutamination. The investigators purified transglutaminase from regenerating optic nerves of fish and demonstrated its capacity for mediating dimerization [70,71].

Their studies clearly suggest the significance of covalent self-association for IL-2 bioactivity in 2

ways. First, they have demonstrated that self-associated IL-2 occurs in a natural setting, found in culture supernatants of regenerating nerves. Second, these investigators have shown that the covalently self-associated form of the cytokine has differential functional implications in the regeneration of nervous tissue in comparison with monomeric IL-2. Thus, it is likely that covalently self-associated IL-2 plays a physiological role in the nervous system.

Since the cytotoxic effect of IL-2 on oligodendrocytes has been shown to involve the α-chain of the IL-2 receptor complex [72], it seems likely that the IL-2 dimers interact with this chain in mediating its effects. This suggestion is also consistent with the interaction of noncovalently self-associated IL-2 with T cells which has been shown to involve the α-chain [69].

Another group of investigators studying the interaction of the cytokine with nervous system cells have indicated that monomeric IL-2 enhances oligodendrocyte proliferation [73]. The possibility that the covalent dimer acts on these cells by inhibiting binding of the monomer to the receptor complex has not been considered but that mechanism could explain the results obtained thus far.

We have crosslinked human recombinant IL-2 using transglutaminase purified from guinea pig liver (Sigma, St. Louis, MO, USA). Recombinant IL-2 (100 ng) was crosslinked for 18 h at room temperature in a total volume of 0.2 ml with 5 mM $CaCl_2$ and 2 mg/ml of transglutaminase. Bovine serum albumin or polyethylene glycol ($M_r = 1000$ Da) was added to prevent adherence to the test tube. After the incubation, samples were electrophoretically separated by SDS-PAGE using a Bio-Rad Mini-Protean II gel apparatus with a 4–20% gradient gel. After running the separation at 100 V for 1.5 h, we transferred the protein to Immobilon-P membranes. The membranes were blocked with bovine serum albumin and probed with goat anti-IL-2 IgG.

The results (Fig. 1, lanes 3 and 4) indicate that transglutamination readily crosslinks IL-2. The presence of bovine serum albumin (Fig. 1, lane 3) obscures multimers with molecular weights around 67 kDa. The use of polyethylene glycol

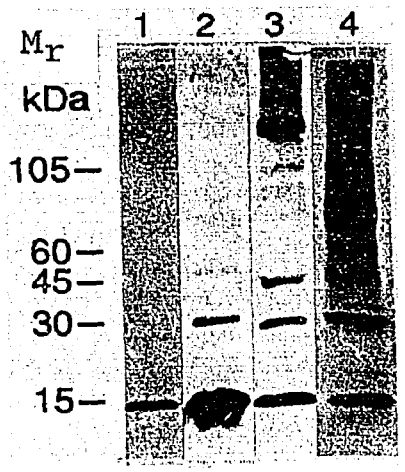

Fig. 1. IL-2 multimers. IL-2 was analyzed by anti-IL-2 IgG blotting after separation via SDS-PAGE. IL-2 diluted in saline is shown in lane 1. IL-2 diluted in water is shown in lane 2. IL-2 crosslinked via transglutamination in the presence of bovine serum albumin (lane 3) or in the presence of polyethylene glycol (lane 4) is shown.

(Fig. 1, lane 4) demonstrates that crosslinking via transglutaminase can readily produce multimers from dimers up to 7-mers.

Fig. 1 also demonstrates that dimers and trimers are spontaneously produced (lane 2). It appears that dilution of the cytokine in water, as opposed to saline, allows for these multimers to be more readily seen. It is possible that dilution in water allows for more cytokine to be delivered to the wells for SDS-PAGE. Water may help to dislodge adherent cytokine monomers and dimers from the sides of the test tube.

It is reasonable to suggest that covalent self-association plays a role in the therapeutic use of the cytokine. The spontaneous formation of covalent dimers indicates that the dimer is given along with the monomer. The potential for IL-2 dimers to initiate differential signalling has been established for oligodendrocytes and may pertain to T lymphocytes and natural killer cells as well.

References

[1] K. Smith, *Science*, 240 (1988) 1169.
[2] S. Gillis and K. Smith, *Nature*, 268 (1977) 154.
[3] D. Morgan, F. Ruscetti and R. Gallo, *Science*, 193 (1976) 1007.

[4] T. Taniguchi, H. Matsui, T. Fujita, C. Takaoka, N. Kashima, R. Yoshimoto and J. Hamuro, *Nature*, 302 (1983) 305.
[5] R. Devos, G. Plaetinck, H. Cheroutre, G. Simons, W. Degrave, J. Tavernier, E. Remant and W. Fiers, *Nucl. Acids Res.*, 11 (1983) 4307.
[6] S. Clark, S. Arya, F. Wong-Staal, M. Kobayashi, R. Kay, R. Kaufman, E. Brown, C. Shoemaker, T. Copeland, S. Oroszlan, K. Smith, M. Sarngadharan, S. Lindner and R. Gallo, *Proc. Natl. Acad. Sci. USA*, 81 (1984) 2543.
[7] S. Rosenberg, E. Grimm, M. McGrogan, M. Doyle, E. Kawasaki, K. Koths and D. Marks, *Science*, 223 (1984) 1412.
[8] G. Smith, G. Ju, B. Ericson, J. Moschera, H. Lahm, R. Chizzonite and M. Summers, *Proc. Natl. Acad. Sci. USA*, 82 (1985) 8404.
[9] D. Williams, D. Regier, D. Akiyoshi, F. Genbauffe and J. Murphy, *Nucl. Acids Res.*, 16 (1988) 10453.
[10] E. Bender, K. Koller and J. Engels, *Gene*, 86 (1990) 227.
[11] T. Williams, J. Burlein, S. Ogden, L. Kricka and J. Kant, *Anal. Biochem.*, 176 (1989) 28.
[12] B. Cullen, *DNA*, 7 (1988) 645.
[13] A. Gulino, A. Vacca, A. Farina, I. Screpanti, M. Maroder, A. Gismondi, A. Santoni, L. Frati, J. Luethy and N. Holbrook, *Biochim. Biophys. Acta*, 1087 (1990) 7.
[14] G. Trinchieri, M. Matsumoto-Kobayashi, S. Clark, J. Seehra, L. London and B Perussia, *J. Exp. Med.*, 160 (1984) 1147.
[15] E. Grimm, A. Mazumder, H. Zhang and S. Rosenberg, *J. Exp. Med.*, 155 (1982) 1823.
[16] B. Vose and G. Bonnard, *J. Immunol.*, 130 (1983) 687.
[17] R. Dempsey, C. Dinarello, J. Meir, L. Rosenwasser, M. Allegretta, T. Brown and D. Parkinson, *J. Immunol.*, 129 (1982) 2504.
[18] F. Erard, P. Corthesy, K. Smith, W. Tiers, A. Conzelmann and M. Nabholz, *J. Exp. Med.*, 160 (1984) 584.
[19] S. Wee, A. Ochoa and F. Bach, *J. Immunol.*, 134 (1985) 310.
[20] M. Lotze, A. Chang, C. Seipp, C. Simpson, J. Vetto and S. Rosenberg, *JAMA*, 256 (1986) 3117.
[21] S. Rosenberg, M. Lotze, L. Muul, A. Chang, F. Avis, S. Leitman, W. Linehan, C. Robertson, R. Lee, J. Rubin, C. Seipp, C. Simpson and D. White, *New Engl. J. Med.*, 316 (1987) 889.
[22] W. West, K. Tauer, J. Yannelli, G. Marshall, D. Orr, G. Thurman and R. Oldham, *New Engl. J. Med.*, 316 (1984) 898.
[23] S. Rosenberg, B. Packard, P. Aebersold, D. Solomon, S. Topalian, S. Toy, P. Simon, M. Lotze, J. Yang, C. Seipp, C. Simpson, C. Carter, S. Bock, D. Swartzentruber, J. Wei and D. White, *New Engl. J. Med.*, 319 (1988) 1676.
[24] M. Papa, J. Mule and S. Rosenberg, *Cancer Res.*, 46 (1986) 4973.
[25] R. Lafreniere and S. Rosenberg, *Cancer Res.*, 45 (1985) 3735.
[26] R. Kozak, H. Galski, L. Jones, R. Puri, M. Willingham, T. Malek, D. Fitzgerald, T. Waldmann and I. Pastan, *J. Immunol.*, 145 (1989) 2766.
[27] M. Ogata, H. Galski, D. Fitzgerald and I. Pastan, *J. Immunol.*, 141 (1988) 4224.
[28] H. Galski, L. Barrett, R. Kirkman, M. Ogata, M. Willingham, D. Fitzgerald and I. Pastan, *Proc. Natl. Acad. Sci. USA*, 86 (1989) 1008.
[29] P. Bacha, S. Forte, S. Perper, D. Trentham and J. Nichols, *Eur. J. Immunol.*, 22 (1992) 1673.
[30] V. Kelley, P. Bacha, O. Pankewycz, J. Nichols, J. Murphy and T. Strom, *Proc. Natl. Acad. Sci. USA*, 85 (1988) 3980.
[31] G. Kaplan, W. Britton, G. Hancock, W. Theuvenet, K. Smith, C. Job, P. Roche, A. Molloy, R. Burkhardt, J. Barker, H. Pradhan and Z. Cohn, *J. Exp. Med.*, 173 (1991) 993.
[32] H. Akuffo, G. Kaplan, R. Kiessling, S. Teklemariam, M. Dietz, J. McElrath and Z. Cohn, *J. Infect. Dis.*, 161 (1990) 775.
[33] M. Reddehase, W. Mutter and U. Koszinowski, *J. Exp. Med.*, 165 (1987) 650.
[34] J. Goronzy, C. Weyand, J. Quan, G. Fathman and P. O'Hanley, *J. Immunol.*, 142 (1989) 1134.
[35] L. Bermudez, P. Stevens, P. Kolonoski, M. Wu and L. Young, *J. Immunol.*, 143 (1989) 2996.
[36] R. Pahwa, T. Chatila, S. Pahwa, C. Paradise, N. Day, R. Geha, S. Schwartz, H. Slade, N. Oyaizu and R. Good, *Proc. Natl. Acad. Sci. USA*, 86 (1989) 5069.
[37] N. Flomenberg, K. Welte, R. Mertelsmann, N. Kernan, N. Ciobanu, S. Venuta, S. Feldman, G. Kruger, D. Kirkpatrick, B. Dupont and R. O'Reilly, *J. Immunol.*, 130 (1983) 2644.
[38] G. Kaplan, W. Britton, G. Hancock, W. Theuvenet, K. Smith, C. Job, P. Roche, A. Molloy, R. Burkhardt, J. Barker, H. Pradhan and Z. Cohn, *J. Exp. Med.*, 173 (1991) 993.
[39] H. Teppler, G. Kaplan, K. Smith, A. Montana, P. Meyn and Z. Cohn, *J. Exp. Med.*, 177 (1993) 483.
[40] S. Rosenberg, M. Lotze, L. Muul, A. Chang, F. Avis, S. Leitman, W. Linehan, C. Robertson, R. Lee, J. Rubin, C. Seipp, C. Simpson and D. White, *New Engl. J. Med.*, 316 (1987) 889.
[41] W. Urba, R. Steis, D. Longo, W. Kopp, A. Munish, L. Marcon, D. Nelson, H. Stevenson and J. Clark, *Cancer Res.*, 50 (1990) 185.
[42] K. Margolin, A. Rayner, M. Hawkins, M. Atkins, J. Dutcher, R. Fisher, G. Weiss, J. Doroshow, H. Jaffe, M. Roper, D. Parkinson, P. Wiernik, S. Creekmore and D. Boldt, *J. Clin. Oncol.*, 7 (1989) 484.
[43] C. Flexner, A. Hugin and B. Moss, *Nature*, 330 (1987) 259.
[44] I. Ramshaw, M. Andrew, S. Phillips, D. Boyle and B. Coupar, *Nature*, 329 (1987) 545.
[45] B. Gansbacher, K. Zier, B. Daniels, K. Cronin, R. Bannerji and E. Gilboa, *J. Exp. Med.*, 172 (1990) 1217.

[46] G. Cortesina, A. DeStefani, M. Giovarelli, M. Barioglio, G. Cavallo, C. Jemma and G. Forni, *Cancer*, 62 (1988) 2482.
[47] G. Forni, M. Giovarelli, A. Santoni, A. Modesti and M. Forni, *J. Immunol.*, 138 (1987) 4033.
[48] R. Steis, M. Bookman, J. Clark, W. Urba, J. McKnight, J. Smith, C. Schoesberg, R. Ozols, R. Young, A. Maluish, S. Beckner and D. Longo, *Proc. Am. Soc. Clin. Oncol.*, 6 (1987) 250.
[49] K. Yasumoto, K. Miyazaki, A. Nagashima, T. Ishida, T. Kuda, T. Yano, K. Sugimachi and K. Nomot, *Cancer Res.*, 47 (1987) 2184.
[50] E. Crum and D. Kaplan, *Cancer Res.*, 51 (1991) 875.
[51] P. Anderson, E. Katsanis, A. Leonard, D. Schow, C. Loeffler, M. Goldstein and A. Ochoa, *Cancer Res.*, 50 (1990) 1853.
[52] E. Abraham and S. Shah, *J. Immunol.*, 149 (1992) 3719.
[53] R. Ho, R. Burke and T. Merigan, *Vaccine*, 10 (1992) 209.
[54] T. Utsugi, C. Dinney, J. Killion, D. Brown and I. Fidler, *Lympho. Cyto. Res.*, 10 (1991) 487.
[55] P. Anderson, E. Katsanis, S. Sencer, D. Hasz, A. Ochoa and B. Bostrom, *J. Immunother.*, 12 (1992) 19.
[56] M. Joffret, S. Morgeaux, C. Laclerc, D. Oth, C. Zanetti, P. Sureau and P. Perrin, *Vaccine*, 8 (1990) 385.
[57] S. Hinuma, M. Hazama, A. Mayumi and Y. Fujisawa, *FEBS Lett.*, 288 (1991) 139.
[58] H. Fell, M. Gayle, L. Grosmaire and J. Ledbetter, *J. Immunol.*, 146 (1991) 2446.
[59] S Gillis, M. Ferm, W. Ou and K. Smith, *J. Immunol.*, 120 (1978) 2027.
[60] C. Bergmann, B. Landmeier and D. Kaplan, *Mol. Immunol.*, 28 (1991) 99.
[61] M. Knauf, D. Bell, P. Hirtzer, Z. Luo, J. Young and N. Katre, *J. Biol. Chem.*, 263 (1988) 15064.
[62] N. Katre, *J. Immunol.*, 144 (1990) 209.
[63] N. Katre, M. Knauf and W. Laird, *Biochemistry*, 84 (1987) 1487.
[64] H. Moon, E. Olsen and J. Nachtman, *Biotech. Ther.*, 1 (1989) 203.
[65] L. Balemans, V. Mattijssen, P. Steerenberg, B. Van-Driel, P. DeMulder and W. Otter, *Canc. Immunol. Immunother.*, 37 (1993) 7.
[66] T. Arakawa, T. Boone, J. Davis and W. Kenney, *Biochem.*, 25 (1986) 8274.
[67] J. Fleischmann, D. Wentworth, F. Valencic, A. Imbembo and K. Koehler, *Biochem. Biophys. Res. Commun.*, 152 (1986) 879.
[68] D. Kaplan, *Mol. Immunol.*, 28 (1991) 1255.
[69] C. Bergmann, D. Gould and D. Kaplan, *Cytokine*, 4 (1992) 192.
[70] S. Eitan, R. Zisling, A. Cohen, M. Belkin, D. Hirschberg, M. Lotan and M. Schwartz, *Proc. Natl. Acad. Sci. USA*, 89 (1992) 5442.
[71] S. Eitan and M. Schwartz, *Science*, 261 (1993) 106.
[72] R. Santeo, A. Altman, R. Knobler, H. Johnson and J. De Vellis, *Proc. Natl. Acad. Sci. USA*, 83 (1986) 9221.
[73] N. Benveniste and J. Merrill, *Nature*, 321 (1986) 610.