Vol. 139, No. 1, 1986
August 29, 1986

BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
Pages 367-374

# The HTLV-III Envelope Protein Contains a Hexapeptide Homologous to a Region of Interleukin-2 That Binds to the Interleukin-2 Receptor

Douglas A. Weigent*, Paul D. Hoeprich†, Kenneth L. Bost*, Terence K. Brunck†, Walter E. Reiher, III†, and J. Edwin Blalock*‡

*Department of Physiology and Biophysics,
University of Alabama at Birmingham,
Birmingham, AL 35294

†Triton Biosciences, Inc.,
1501 Harbor Bay Parkway,
Alameda, CA 94501

Received July 18, 1986

A region of human interleukin-2 (IL-2) which was predicted to be a contact point with its receptor was used to locate a homologous region in the envelope protein of human T-lymphotropic retrovirus (HTLV-III). This homologous six amino acid peptide from the carboxy (C)-terminus of the HTLV-III envelope protein was found to inhibit the biological activity of human IL-2 in a murine spleen cell proliferation assay. When conjugated to a carrier protein, this peptide inhibited the binding of radiolabelled IL-2 to its receptor. The biological activity of the peptide was antagonized by a six amino acid peptide fragment of the IL-2 receptor which was predicted to be the contact point on the receptor that corresponded to the binding region of IL-2. The HTLV-III peptide also inhibited the binding of radiolabelled IL-2 to polyclonal anti-IL-2 antiserum. These data support the previous assignment of contact points between IL-2 and its receptor. They also suggest two possible mechanisms of immunosuppression during acquired immunodeficiency syndrome (AIDS). One involves direct competition of the envelope protein or its fragments with IL-2 for binding to the IL-2 receptor. The other involves antibodies to the envelope protein which crossreact with and neutralize IL-2. 

According to a newly proposed theory of molecular recognition, two peptides representing potential translations of complementary nucleic acid sequences should bind to each other (1-3). This hypothesis was tested by preparing synthetic peptide counterparts (termed complementary peptides) specified by RNA sequences complementary to the mRNA sequences for several hormones, including corticotropin (ACTH), $\gamma$-endorphin and luteinizing hormone releasing hormone (LHRH) (1,4-6). These complementary peptides bound their respective hormones with high affinity and specificity in solid phase assays, suggesting that the

‡To whom correspondence should be addressed.

0006-291X/86 $1.50

synthetic peptides resemble receptor binding sites. Consistent with this idea was the finding that antibodies to the complementary peptides were able to bind their respective hormone receptors (1,4-6).

Further support for the concept that complementary peptide sequences may be related to receptor binding sites was found in the comparison of the mRNA sequences of interleukin-2 (IL-2), epidermal growth factor (EGF), and transferrin with their respective receptor sequences (7). Specifically in the case of IL-2, a sequence complementary to the IL-2 receptor contained two regions of high nucleotide homology, and thus high amino acid homology, with IL-2. One of these regions (a pentapeptide) was in the signal sequence of IL-2, whereas the other region (a hexapeptide) was in the body of the IL-2 molecule (residues 34-39). Based on the proposed molecular recognition theory, the hexapeptide region within the body of the IL-2 molecule is predicted to be the binding site of IL-2 with its receptor. If correct, the theory predicts that synthetic peptides corresponding to the hexapeptide region of complementarity between IL-2 and its receptor would inhibit the binding of IL-2 to its receptor, and that peptides corresponding to the pentapeptide region of complementarity would not influence binding. Furthermore, a significant amino acid homology between the proposed hexapeptide binding region of IL-2 and the HTLV-III envelope protein was found (Fig. 1). Also observed were similarities between this hexapeptide region and envelope proteins of other immunosuppressive retroviruses (8-14). In the present report, we have tested the hypothesis that the hexapeptide region of the HTLV-III envelope protein that was identified by homology with the proposed receptor binding site on IL-2 is able to interfere with IL-2 binding to its receptor and inhibit biological activity. Further, we have examined the possible crossreactivity between antibodies to IL-2 and the hexapeptide. The results suggest two possible mechanisms for immunosuppression during AIDS and provide additional support for the molecular recognition theory that led to the discovery.

## MATERIALS AND METHODS

**Peptides.** All peptides were synthesized by the Merrifield solid phase technique and were purified with reversed phase high performance liquid

chromatography (HPLC). Peptides were greater than 95% pure, and sequences were verified by amino acid composition.

**Interleukin-2 Bioassay.** C57/B16 spleen cells ($4x10^6$/ml) were cultured for 4 days at 37°C in humidified 5% $CO_2$ in T-75 tissue culture flasks with concanavalin A (10 μg/ml). Culture medium was RPMI 1640 supplemented with 10% fetal bovine serum and penicillin (100 U/ml), streptomycin (100 μg/ml) and mycostatin (100 U/ml). After 4 days, the blast cells were harvested, washed three times by centrifugation, and resuspended at a concentration of $4x10^6$/ml. Cells were incubated for 1h in medium to remove endogenous IL-2. Two-fold serial dilutions of peptides were made in 0.1 ml volumes of medium in 96 well flat bottom microtiter plates. One-tenth ml of blast cell suspension was added to each well followed by 10 units/ml of HPLC purified natural human IL-2 (Collaborative Research, Inc., Lexington, MA) and the cultures incubated for a further 24h. One μCi $^3$H-methyl thymidine (58 Ci/mmol) (New England Nuclear, Boston, MA) was added to each well and cultures incubated for an additional 24h. The labelled cells were harvested on Gelman glass fiber filters after TCA precipitation. The samples were counted in a liquid scintillation counter.

**Interleukin-2 Radioreceptor Assay.** Spleen cells were cultured as described above, except that washed spleen cells (100,000) were added to each well containing various concentrations of peptides alone or mixtures of peptides and homogeneous recombinant human $^{125}$I-IL-2 ($10^{-11}$ M, 40 μCi/μg, NEN Research Products, Boston, MA) and incubated for 30 min at room temperature. The cells were washed three times by centrifugation at 1,600 rpm for 5 min at 4°C and cell associated radioactivity was determined on a TM Analytic gamma counter.

**Interleukin-2 Immunoassay.** Rabbit polyclonal antibody to homogeneous recombinant human IL-2 (Collaborative Research, Lexington, KY), which has a virtually identical titer against natural IL-2, was plated onto polycarbonate microtiter plates at 5 units/well and was incubated overnight at 4°C. Plates were then washed three times with PBS containing Tween (0.05%). The plates were incubated for 2h at room temperature with $^{125}$I-IL-2 ($10^{-11}$ M, 40 μCi/μg) in the absence or presence of nonlabeled IL-2 (25U) or the indicated synthetic peptides at 20 or 80 μg/ml. The wells were washed three times for 5 minutes at 4°C and bound radioactivity was determined on a TM Analytic gamma counter.

## RESULTS

The amino acid sequences for the homologous regions of the HTLV-III envelope protein and IL-2, the penta- and hexapeptide regions of the IL-2 receptor, and the synthetic peptides examined in this study are shown in Figure 1. As shown in Table 1, the addition of the HTLV-III hexapeptide (peptide 1, the IL-2 homologue) caused a dose-dependent decrease in the ability of natural IL-2 to stimulate murine spleen cell proliferation. A larger 16 amino acid fragment of the envelope protein (peptide 2), selected from the conserved C-terminus, lacked IL-2 inhibitory activity. Though peptide 2 contained the entire sequence of peptide 1, the conformation of peptide 2 was evidently not compatible with receptor binding. That peptide conformation may be critical in such assays is also suggested by previous work wherein a retroviral envelope protein fragment (CKS-17) was active only when coupled to

| *Name* | *Sequence* | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *Naturally-occurring sequences* | | | | | | | | | | | | | | | |
| IL-2 hexamer | | | | | | | | | | | Leu (34) | Glu | His | Leu | Leu | Leu (39) |
| HTLV-III C-terminus | Arg (841) | His | Ile | Pro | Arg | Arg | Ile | Arg | Gln | Gly | Leu | Glu | Arg | Ile | Leu | Leu (856) |
| IL-2 receptor hexamer | | | | | | | | | | | Glu (1) | Leu | Cys | Asp | Asp | Asp (6) |
| IL-2 receptor pentamer | Met (18) | Ala | Tyr | Lys | Glu (22) | | | | | | | | | | | |
| | *Synthetic peptide sequences* | | | | | | | | | | | | | | | |
| Peptide 1 | | | | | | | | | | | Leu | Glu | Arg | Ile | Leu | Leu |
| Peptide 2 | Arg | His | Ile | Pro | Arg | Arg | Ile | Arg | Gln | Gly | Leu | Glu | Arg | Ile | Leu | Leu |
| Peptide 3 | | | | | | | | | | | Glu | Leu | Met | Asp | Asp | Asp |
| Peptide 4 | Met | Ala | Tyr | Lys | Glu | | | | | | | | | | | |

Numbers under the amino acid residues indicate the residue in the native proteins. Peptide 1 is identical to the HTLV-III envelope peptide and analogous to the IL-2 sequence. Peptide 2 is the same sequence as peptide 1 with the addition of the 10 amino acids immediately upstream of the hexamer in the HTLV-III envelope protein. Peptide 3 corresponds to the sequence which is in the interleukin-2 receptor and is complementary to peptide 1, while peptide 4 is an IL-2 receptor sequence that is complementary to the IL-2 signal sequence (7). For peptide 3 which corresponds to the first IL-2 receptor sequence, a Met was substituted for Cys to prevent any disulfide bonding.

**Figure 1.** Amino acid sequences for the synthetic peptides from the region of homology between the HTLV-III envelope protein and interleukin-2 and from complementary sequences in the interleukin-2 receptor.

Table 1
Inhibition of IL-2 Dependent Murine Spleen Cell Proliferation

| Peptide | Concentration of Peptide (μg/ml) | | | | |
|---|---|---|---|---|---|
| | 0 | 10 | 20 | 40 | 80 |
| | Percent inhibition of control[a] | | | | |
| 1 | 0 | 12 ± 2 | 31 ± 5 | 56 ± 5 | 69 ± 8 |
| 2 | 0 | 3 ± 1 | 3 ± 2 | 1 ± 1 | 4 ± 3 |
| 4 | 0 | 2 ± 2 | 2 ± 1 | 5 ± 3 | 3 ± 2 |
| 1 (80 μg) + 3[b] | 69 ± 8 | N.D.[c] | 53 ± 6 | 39 ± 4 | 30 ± 5 |

[a] Two-fold serial dilutions of peptides were made in 0.1 ml volumes of culture medium in 96 well flat bottom microtiter plates. One-tenth ml of blast cell suspension was added to each well followed by 10 units/ml of HPLC purified natural human IL-2 and the assay was then performed as described in Materials and Methods. Values represent the mean percent inhibition ± S.E.M. of triplicate samples. Cultures grown in the absence of IL-2 incorporated 5954 CPM per well, whereas IL-2 treated cultures incorporated 26,957 cpm. The results shown are typical results from one experiment performed five times.

[b] For the mixed peptide sample, peptide 3 at the concentrations indicated in the figure were combined with peptide 1 (80 μg/ml) and were incubated for 30 min at 4°C prior to the assay for IL-2 activity.

[c] N.D. = not done.

carrier protein (14). Inhibition of IL-2 activity by peptide 1 was not the result of a cytotoxic effect since cells incubated with this peptide had the same viability as controls (>80%) as determined by trypan blue dye exclusion. The inhibitory action of peptide 1 (the IL-2 homologue) was reduced by preincubation with peptide 3, the corresponding IL-2 receptor (complementary) sequence. Another IL-2 receptor fragment, peptide 4, was inactive alone (Table 1) and when preincubated with peptide 1 (data not shown). These data suggest that peptides 1 and 3 are interacting and that they represent the critical binding regions of the hexapeptide (and, by implication, IL-2) and the IL-2 receptor.

To determine whether the inhibitory effect of peptide 1 occurred as a result of the blocking of IL-2 binding to its receptor, radioreceptor assays were performed. Preliminary experiments indicated that peptide 1 alone had only marginal ability to block $^{125}$I-IL-2 binding to its receptor (data not shown). This suggested that while peptide 1 could bind the receptor and block an IL-2 generated biological signal, it was not sufficiently large to completely block IL-2 binding. This possibility was addressed by attempting to sterically hinder the receptor by coupling the peptide to a bulky protein. As shown in Table 2, peptide 1 coupled to bovine serum albumin (BSA) caused marked inhibition of binding of IL-2 to its receptor, whereas other peptides were essentially inactive. Addition of the putative IL-2 receptor binding region (peptide 3) to the peptide 1 conjugate inhibited the effect of peptide 1. Consistent with earlier results, another IL-2 receptor sequence (peptide 4) did not block the activity of peptide 1 conjugate. The activity of peptide conjugate 1 and the interaction of peptide 3 with peptide 1 conjugate are consistent with the results of the proliferation assay and confirm the expectations of the molecular recognition theory.

To evaluate whether the IL-2 homologue in the envelope protein represents a major IL-2 epitope, the following experiments were performed. As is shown in Table 3, peptide 1, but no other peptides, inhibited the binding of $^{125}$I-IL-2 to polyvalent antibodies to recombinant human IL-2 up to 49% in comparison to 95%

Table 2
Inhibition of $^{125}$I-IL-2 Binding to Murine Spleen Cells

| Peptide[a] | Concentration of Peptides (μg/ml) or IL-2 (U/ml) | | |
|---|---|---|---|
| | 25 U/ml | 20 μg/ml | 80 μg/ml |
| | Percent inhibition of control specific binding[b] | | |
| Nonlabeled IL-2 | 95 ± 5 | | |
| BSA-1 | | 42 ± 9 | 78 ± 7 |
| BSA-2 | | 0 | 0 |
| BSA-3 | | 8 ± 4 | 36 ± 3 |
| BSA-1 + 3 | | 0 | 7 ± 1 |
| BSA-1 + 4 | | 48 ± 11 | 82 ± 9 |

[a]Peptides were conjugated to BSA using the crosslinker, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide as previously described (14).

[b]The IL-2 radioreceptor assay was performed as described in the Materials and Methods. The indicated concentrations of peptides, or mixtures of peptides and homogeneous recombinant human $^{125}$I-IL-2 were incubated with splenic blast cells for 30 minutes at room temperature. Total counts bound were $2.9 \times 10^3$ of which 95% could be blocked by nonlabeled IL-2. To prevent nonspecific binding, all assays were performed in the presence of BSA (10 μg/ml). The results are expressed as the mean percent inhibition of control specifically bound counts ($2.76 \times 10^3$ CPM) ± S.E.M. The results are typical from three separate experiments.

Table 3

Inhibition of $^{125}$I-IL-2 Binding to Antibodies to IL-2

| Peptide | Concentration (μg/ml) | |
|---|---|---|
| | 20 | 80 |
| | Percent Inhibition of Specific Binding[a] | |
| 1 | 22 ± 6 | 49 ± 8 |
| 2 | 3 ± 2 | 4 ± 2 |
| 3 | 3 ± 3 | 7 ± 4 |
| 4 | 5 ± 4 | 1 ± 1 |

[a]IL-2 immunoassay was performed as described in the Materials and Methods section in the presence or absence of the indicated peptide concentrations. The results are presented as the percent inhibition of the total specific IL-2 binding ($1.4 \times 10^4$ CPM) to its antibody ± S.E.M. of three replicates. Ninety-five percent inhibition was observed with saturating nonlabeled IL-2. The results are typical of three separate experiments.

inhibition by saturating nonlabeled IL-2. Thus, peptide 1 must resemble a major epitope within the IL-2 molecule, and it is recognized by a large fraction of the antibodies. Though the lack of binding of peptide 2 to antibodies to IL-2 is not understood, it is expected to be related to the conformation of this peptide.

## DISCUSSION

These studies have both practical and theoretical implications. Practically, they suggest two possible means by which HTLV-III might cause significant immunosuppression, even though in many cases only a small fraction of lymphocytes is infected. This would occur when antibodies directed against the peptide 1 epitope of the envelope protein crossreact with and neutralize IL-2 activity. After initial viral infection, any additional endogenous or "therapeutic" IL-2 could act as a booster for the expression of such antibodies. AIDS sera are currently being screened by immunoassay for the presence of antibodies to peptide 1 and IL-2, and 17 out of 19 individuals were positive, while normal sera were negative (manuscript in preparation). The presence of such antibodies would suggest several therapeutic regimens such as plasmaphoresis to remove anti-IL-2 antibodies or selective neutralization of such antibodies in vivo. Another possible mechanism for immunosuppression could be the action of the envelope protein or fragments containing the active hexapeptide region as IL-2 antagonists. Therapeutic treatments based on the removal or neutralization of such active molecules could also be attempted. For example, vaccines directed against the C terminal region (excluding the terminal 6 amino acids) of the HTLV-III envelope protein may prove effective (8).

Theoretically, these results provide additional strong support for the hypothesis that ligand and receptor binding sites are identified by complementary segments of nucleic acids and that complementary peptide molecular recognition is an integral part of the genetic code (1-3). This idea is substantiated by the ability of the theory to identify a contact point between IL-2 and its receptor by simple sequence analysis. Thus, interacting (complementary) peptides are directly associated with the complementarity of

nucleic acids. Also implicit in the theory is the corollary that for each receptor binding site, there exists the information for a potential endogenous ligand defined by the complementary nucleotide sequence of the site. These ideas may, in time, provide a unifying hypothesis and a simplified understanding for the chemical basis of interacting biological systems such as cellular communication, ontological development, differentiation, and, as a result of current work, host-parasite relations.

## ACKNOWLEDGEMENTS

We thank Drs. J. D. Neill, G. Shaw, and B. Hahn for helpful scientific discussions, and D. Weigent for secretarial assistance. This research was supported by a grant from Triton Biosciences, Inc., Alameda, CA.

## REFERENCES

1. Bost, K.L., Smith, E.M., and Blalock, J.E. (1985) Proc. Natl. Acad. Sci. USA 82, 1372-1375.
2. Blalock, J.E., and Smith, E.M. (1984) Biochem. Biophys. Res. Comm. 121, 203-207.
3. Blalock, J.E., and Bost, K.L. (1986) Biochem. J. 234, 679-683.
4. Bost, K.L., and Blalock, J.E. (1986) Molec. Cell. Endocrinol. 44, 1-9.
5. Carr, D.J.J., Bost, K.L., and Blalock, J.E. (1986) J. Neuroimmunol., in press.
6. Mulchahey, J.J., Neill, J.D., Dion, L.D., Bost, K.L., and Blalock, J.E. (1986) Proc. Natl. Acad. Sci. USA, in press.
7. Bost, K.L., Smith, E.M., and Blalock, J.E. (1985) Biochem. Biophys. Res. Comm. 128, 1373-1380.
8. Reiher, W.E. III, Blalock, J.E., and Brunck, T.K. (1986) Proc. Natl. Acad. Sci. USA, submitted.
9. Wain-Hobson, S., Sonigo, P., Panos, O., Cole, S., and Alizon, M. (1985) Cell 40, 9-17.
10. Crowl, R., Ganguly, K., Gordon, M., Conroy, R., Schaber, M., Kramer, K., Shaw, G., Wong-Staal, F., and Reddy, E.P. (1985) Cell 41, 979-986.
11. Rather, L., Haseltine, W., Patarca, R., Livak, K.J., Starcich, B., Josephs, S.F., Doran, E.R., Rafalski, J.A., Whitehorn, E.A., Baumeister, K., Ivanoff, L., Petteway, Jr., S.R., Pearson, M.L., Lautenberger, J.A., Papas, T.S., Ghrayeb, J., Chang, N.T., Gallo, R.C., and Wong-Staal, F. (1985) Nature 313, 277-284.
12. Muesing, M.A., Smith, D.H., Cabradilla, C.D., Benton, C.V., Lasky, L.A., and Capon, D.J. (1985) Nature 313, 450-458.
13. Sanchez-Pescador, R., Power, M.D., Barr, P.J., Steimer, K.S., Stempien, M.M., Broun-Shimer, S.L., Gee, W.W., Randolph, A.R.A., Levy, J.A., Dina, D., and Luciu, P.A. (1985) Science 227, 484-492.
14. Cianciolo, G.J., Copeland, T.D., Oroszlan, S., and Snyderman, R. (1985) Science 230, 453-455.