IL-2 DERIVATIVES POLYPEPTIDES WITH AGONIST ACTIVITY FOR THERAPY OF CANCER AND CHRONIC INFECTIONS.

Scope of the invention.

The present invention relates to immunology. It relates specifically to the therapeutic modulation of the immune system by means of natural molecule analogues that have the agonistic action of the original molecule but, unexpectedly, showed a superior therapeutic efficacy.

Background of the invention:

Interleukin 2 (IL-2) was first growth factor described for T cells. Since its discovery it was observed its ability to promote proliferation and survival of T *in vitro* cells (Smith, KA. (1988) Science. 240, 1169-76), as well as to boost immune response in the context of T viral infections (Blattman, JN, et al. (2003) Nat Med 9, 540-7) or vaccines (Fishman, M., *et al.* (2008) J Immunother. 31, 72-80, Kudo-Saito, C ., *et al.* (2007) Cancer Immunol Immunother. 56, 1897-910 ; Lin, CT., *et al.* (2007) Immunol Lett. 114, 86-93). However, this classical role of IL-2 as a promoter of T immune response has been questioned recently by numerous experimental data (Almeida, AR., et *al.* (2002) J Immunol. 169, 4850-60 ; de la Rosa, M., *et al.* (2004) Eur J Immunol. 34, 2480-8, Malek; TR, *et al.* (2004) Nat Rev Immunol. 4, 665-74) showing that this cytokine is an homeostatic growth factor for natural regulatory T cells CD4 + CD25 +FoxP3 +(Tregs). Interleukin-2 has also been proposed as a major player in the mechanism by which regulatory T cells suppress the activity and expansion of other effector cells such as CD4 T helper cells, cytotoxic CD8 T cells and natural killer (NK) cells. In particular it has been recently proposed that regulatory T cells suppress other T cells, inducing the local decrease in the levels of IL-2 (Pandiyan, P., *et al.* (2007) Nat Immunol. 8, 1353-1362). This suppressive effect is based on: a) their ability to directly inhibit that effector T cells produce new IL-2 (Almeida, AR., *et al.* (2002) J Immunol. 169, 4850-60; Takahashi, T., et *al.* (1998) Int Immunol. 10, 1969-80; Thornton, AM., *et al.* (1998) J Exp Med 188, 287-96; Wolf, M., *et al.* (2001) Eur J Immunol. 31, 1637-45); b) their ability to sequester, internalize and degrade quickly the IL-2 present in their microenvironment (Pandiyan, P., *et al.* (2007) Nat Immunol. 8, 1353-62) and c) its ability to over-express the alpha chain of the IL-2 receptor (Kuniyasu, Y., *et al.* (2000) Int Immunol. 12, 1145-1155), which allows for a more efficient use of IL-2 when its concentrations are low.

In summary, IL-2 is a highly pleiotropic cytokine which is very relevant in the biological activity of different cell populations. This property makes the IL-2 an important node in the regulation of the immune response, making it an attractive target for therapies and complex immune modulation.

IL-2 has been used for several years in cancer therapy. In particular its use at high doses is an approved therapy in several countries for the treatment of metastatic melanoma and renal cell carcinoma. However, the direct use of IL-2 in patients is severely limited by its toxic effects and low efficacy. So much so that only 20% of eligible patients receive IL-2 based therapy and only 17% of those patients treated show an objective response. A likely explanation for this dramatic failure in the clinic setting is that therapy with native IL-2 also stimulates populations of regulatory T cells (Ahmadzadeh, M., et *al.* (2006) Blood. 107, 2409-14) which dampen the desired immuno-stimulation. Nowadays large amounts of pre-clinical evidence support this idea. In particular, experiments in murine models show that the primary activity of the IL-2 injected *in vivo* is the homeostatic expansion of natural regulatory T cells.

Several strategies have been developed to mitigate the toxic effects of IL-2 therapy. Some of these strategies are based on the use of mutated variants of IL-2, designed to increase the capacity of this molecule to signal mainly by the high affinity receptor (alpha, beta and gamma chains) and not by the intermediate affinity receptor (beta and gamma chains). The basic idea is to promote signaling in T cells instead of signaling in NK cells, which are believed to be responsible for the observed toxic effects. The following inventions are in this line of work: U.S. Pat. 7,186,804, U.S. Pat. 7,105,653, U.S. Pat. 6,955,807, U.S. Pat. No. 5,229,109, U.S. Patent Application 20050142106. It is important to note that none of these inventions relates to muteins of IL-2 that have greater therapeutic efficacy than the *in vivo* native IL-2, based on their decreased ability to stimulate natural regulatory T cells.

Other mutated variants of IL-2 have been created with the aim of increasing their pharmacological activity by, for example, improving its folding or increasing their lifetime in blood. Among others, the following inventions are in this line of work: U.S. Pat. No. 4,959,314, U.S. Pat. No. 5,116,943, U.S. Pat. No. 4,853,332. Again, none of these muteins have a decreased ability to activate regulatory T cells or shows greater therapeutic efficacy.

Finally, it should be mentioned that in the literature there are numerous proposals of therapeutic agents (Kreitman, R.J. (2009) Curr Pharm Des. 15, 2652-64; Litzinger, M.T., Fernando, R., Curiel, T.J., Grosenbach, D.W., Schlom, J. and Palena, C. (2007) Blood. 110, 3192-201; Morse, M.A., Hobeika, A.C., Osada, T., Serra, D., Niedzwiecki, D., Lyerly, H.K. and Clay, T.M. (2008) Blood. 112, 610-8; Onizuka, S., Tawara, I.,

Shimizu, J., Sakaguchi, S., Fujita, T. and Nakayama, E. (1999) Cancer Res. 59, 3128-33; Quezada, S.A., Peggs, K.S., Curran, M.A. and Allison, J.P. (2006) J Clin Invest. 116, 1935-45) that propose to modulate or reduce the activity of regulatory T cells *in vivo*. These therapeutic agents have been tested in animal models or even in patients as a direct therapy of cancer or to enhance the effect of vaccines. There are also some reports that propose to modulate the activity of IL-2, in particular monoclonal antibodies (Boyman, O., Kovar, M., Rubinstein, M.P., Surh, C.D. and Sprent, J. (2006) Science. 311, 1924-1927; Boyman, O., *et al.* (2006) Expert Opin Biol Ther. 6, 1323-31; Kamimura, D., *et al.* (2006) J Immunol. 177, 306-14; Murakami, M., Sakamoto, A., Bender, J., Kappler, J. and Marrack, P. (2002) Proc Natl Acad Sci USA. 99, 8832-7; Tomala, J., Chmelova, H., Mrkvan, T., Rihova, B. and Kovar, M. (2009) J Immunol. 183, 4904-4912), to promote better or more effective immune responses. However, to our knowledge, there is no report in the literature, based on mutated variants of IL-2, showing the possibility of obtaining greater therapeutic efficacy based on their decreased ability to stimulate natural regulatory T cells.

Brief Description of the Invention

The present invention relates to the production of mutated variants of IL-2, which show greater therapeutic efficacy than the native IL-2 in transplantable murine tumor models. These muteins are characterized by being partial agonists of IL-2 activity and selected due to their particularly low ability to stimulate natural regulatory T cells (T CD4+CD25+FoxP3+) *in vitro* and/or *in vivo*. The *in vivo* therapeutic efficacy of these muteins offers a practical solution to improve IL-2 therapy in malignant tumors. In particular, these muteins will provide a way to overcome the limitations observed in native IL-2 therapy which are derived from their proven ability to expand *in vivo* natural regulatory T cells.

The present invention relates to polypeptides which share their primary sequence with the human IL-2, except for several amino acids that have been mutated. The mutations introduced substantially reduce the ability of these polypeptides to stimulate *in vitro* and *in vivo* regulatory T cells (T CD4+CD25+FoxP3+) and give IL-2 a greater efficacy in the therapy of murine transplantable tumors. The present invention also includes therapeutic uses of these mutated variants, alone or in combination with vaccines for therapy of diseases such as cancer or infections where the activity of regulatory T cells (Tregs) is relevant.

The present invention allows for a substantial improvement of the current strategies of immunomodulation based on IL-2, both for in the direct therapy of cancer and in combination with different vaccines. In particular the replacement of the native IL-2 by

the mutated variants described herein, will prevent the expansion of regulatory T cells which markedly reduce the desired therapeutic effects.

Detailed Description of the Invention.

Obtainment of IL-2 analogue polypeptides.

The present invention relates to polypeptides of 100 to 500 amino acids in lenght, preferably of 140 residues size whose apparent molecular weight is at least 15 kD. These polypeptides maintain high sequence identity, more than 90%, with native IL-2. In a region of their sequence, they include 3 to 6 mutations as compared to the native IL-2. In these positions, these polypeptides are mutated introducing amino acid residues different from those in the same position in the native IL-2. The aminoacids that replace the original residues are selected to have physicochemical properties quite different to the ones of the original amino acid, changing the polar residue for nonpolar, uncharged for charged, large for small, acid for base, among others changes.

The polypeptides of the present invention may be referred to as immunomodulatory polypeptides, IL-2 analogs or IL-2 muteins, among others names. These polypeptides are designed based on the 3D structure of the IL-2 receptor complex (available in PDB public database), introducing mutations mainly in the positions of the IL-2 corresponding to amino acids significantly exposed to the solvent and that are highly conserved in the IL-2 from different species (sequences obtained from Swissprot database). The amino acids exposed to the solvent of the type mentioned above are identified using bioinformatics softwares for visualization of protein structures such as RASMOL, SwissPDBviewer or others. The conserved positions in the sequence of IL-2 were identified using bioinformatics softwares for multiple sequence alignment, e.g., Fasta, ClusterW or others.

The polypeptides of this invention can be obtained by various satrategies, including by protein synthesis. They could also be obtained by genetic engineering techniques, for instance by expressing them in bacteria such as *E. coli* or other bacteria and also in mammalian cells such as NSO cells or other mammalian cells. Point mutations at specific positions may also be obtained by site-directed mutagenesis techniques by means of polymerase chain reaction assay (PCR).

Unexpectedly, the inventors found a substantial advantage of these muteins with respect to the traditional use of native IL-2. This advantage lies in their increased effectiveness in tumor therapy, derived from its ability to prevent the expansion of regulatory T cells.

Selection of the polypeptide analogs of IL-2 in terms of biological activity ;

The polypeptides of the present invention are selected by the following properties:

1) Agonist action of native IL-2. This property can be evaluated directly in *in-vitro* proliferation assays with cell lines dependent on IL-2 such as CTLL2 or Kitt225, or with assays with mixtures of murine and/or human T lymphocytes. These muteins must have a specific stimulatory activity 5 to 50 times less than that of native IL-2 in these trials.

2) Loss of capacity, as compared to native IL-2, to stimulate *in vitro* and/or *in vivo* populations of regulatory T cells. This property can be assessed, for instance, by studying the ability of the muteins of the present invention, as compared to those of native IL-2, to directly induce expansion of T CD4+CD25+ cells, purified from naïve mice and stimulated with an in vitro anti-CD3 antibody. It can be also assessed by injecting, intraperitoneally or subcutaneously, these muteins or the native IL-2 in mice, for five days, and evaluating its effect on the expansion or increase in the rate of proliferation of populations of regulatory T cells (TCD4+CD25+FoxP3+). The activity of the mutated IL-2 on Treg cells must be at least 1000 times lower than that of native IL-2 in these trials.

3) Increased therapeutic effect with respect to the native IL2 in animal models. This property can be assessed, for example, by comparing the antitumor or anti-metastatic effect of the muteins and the native IL-2 as monotherapy in transplantable tumor models (e.g. B16 melanoma). It can also be evaluated through the potentiating effect of the cellular and/or humoral response to a vaccine of interest. The muteins must show greater therapeutic efficacy than the native IL-2 in doses containing an equal total mass of proteins of IL-2 and the mutein.

The present invention is particularly related to the muteins specified on Table 1. These muteins have multiple aminoacids substitutions which give them the above-mentioned properties.

Table 1: Designed Muteins that have the three basic properties described in this patent. The mutations are referred to according to the numbering of the human IL-2.

Mutations
R38K, F42I, Y45N, E62L, E68V

R38K, F42Q, Y45E, E68V
R38A, F42I, Y45N, E62L, E68V
R38K, F42K, Y45R, E62L, E68V
R38K, F42I, Y45E, E68V
R38A, F42A, Y45A, E62A

The present invention also includes additional modifications to the class of IL-2 muteins mentioned above and especially to those described in Table 1. Whether it is to increase their affinity to specific components of the IL-2 receptor, but without affecting or even improving its agonist character that does not stimulate regulatory T cells, or to improve their in vivo pharmacodynamics: increase half-life or reduce their internalization by T cells. These additional mutations may be obtained by rational design with bioinformatics tools, or by using combinatorial molecular libraries of different nature (phage libraries, libraries of gene expression in yeast or bacteria).

In another aspect the present invention relates to a fusion protein comprising any of the immunomodulatory polypeptides described above, coupled to a carrier protein. The carrier protein can be Albumin or the Fc region of human immunoglobulins.

Therapeutic application of IL-2 analog polypeptides;

This invention also includes pharmaceutical compositions comprising as active ingredient the muteins of IL-2 and its analogs, disclosed by means of the present invention and its potential therapeutic applications in order to enhance the natural or vaccine-induced immune response in diseases such as cancer or chronic infections where regulatory T cells are particularly relevant.

For its therapeutic use, the polypeptide of the present invention should be administered to a subject carrier of the disease independently or in combination with other polypeptides or other substances that facilitate or enhance its therapeutic action. The route of administration may be any route of administration described by the state of the art for parenteral administration of drugs. Preferably it should be administered intravenously, intramuscularly, subcutaneously or intratumorally.

The polypeptides described herein may also be administered as part of a pharmaceutical composition that is used in the treatment of cancer and chronic infectious diseases or to enhance the cellular and/or humoral response to vaccines, as replacement of the native IL2. The polypeptides of the present invention can be used in

combination with therapeutic vaccines for cancer or with prophylactic vaccines in infectious diseases where regulatory T cells are relevant.

To obtain the desired therapeutic effect, the polypeptide of the present invention should be administered at doses high enough to ensure its concentration in the peripheral lymph node or in the peripheral site relevant to the disease under study and within the range of concentrations in which the mutein which shows an immune-stimulatory effect. The dose in question should therefore be adjusted according to the type of disease and the route of administration under study. For example in the case of tumor therapy, the dose should be adjusted to achieve concentrations of the mutein inside the tumor and/or in the loco-regional lymph node so as to ensure the stimulation of an antitumor immune response. The dose ranges to explore can range from hundreds of micrograms to hundreds of milligrams per dose. For applications in which the mutein replaces traditional therapy with native IL-2 the mutein dose used should be less than or equivalent in activity (determined using assay with CTLL2 line) to the one traditionally used for native IL-2.

The number of administrations to apply should also be adjusted according to the biodistribution of the mutein in question. In general, the aforementioned effective levels should be maintained for 2 to 30 consecutive days. It should be noted, for example, that if the mutein is coupled to a carrier protein, the frequency of its administration shall be adjusted accordingly. For applications in which the native IL-2 is replaced the scheme of administration of the mutein may be similar to that used in traditional therapy.

Therapeutic action should be understood by, full or partial remission of the symptoms of the disease. In cancer, a decrease in tumor volume or an increase in the time before relapse will be, among others, the criterion of remission of the disease.

The polypeptides of the invention are particularly useful in the therapy of tumors such as melanomas and renal tumors.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1. Obtainment of the mutein. a) Expression of the mutein in strain BL21DE3 of *E.coli* evaluated by electrophoresis in SDS-polyacrylamide gel (SDS-PAGE), lane1: Total proteins of BL21DE3 strain, negative control of expression; lanes 2 and 3: Two examples of the expression levels achieved in this strain, the arrow indicates the band corresponding to the mutein. b) Reverse phase chromatogram showing the main final purification step of the protein, the arrow indicates the peak corresponding to the protein of interest. c) Purification of the mutein assessed by SDS-PAGE 1: Results of

the process of inclusion body isolation, 2: mutein obtained after purification by reverse phase

Figura 2. Assessment of the agonist character of IL-2 mutein. a) Measurement by flow cytometry of the binding ability of the mutein to the surface of the CTLL2 cell line. Both IL-2 and mutein were detected using an anti 6His tag MAb b) The graph shows the ability of the mutein to induce the proliferation of the IL-2- dependent T-cell line CTLL2, compared with native IL-2. The proliferation was measured by MTT incorporation. .

Figure 3. The mutein does not induce *in vitro* proliferation of regulatory T cells. a) Flow cytometry graph showing the purity of CD3+CD4+CD25+ population purified from C57BL/6 mouse lymphonodes. b) Treg cells were stimulated *in vitro* with anti-CD3 mAb and native IL-2 was administered to them at a concentration of 0.5 ng/mL or mutein at a concentration of 32 ng/mL for 72 hours, the graph shows the number of live cells recovered after each treatment compared with the control where no cytokine was added. The selected concentrations correspond to the concentration at which each molecule induces the same proliferation of CTLL2 line.

Figure 4. Evaluation of the effect of treatment with mutein on the proliferation of cell populations. a) Quantification of the relative weights of the spleens of mice treated with the mutein for five days. The weights of the spleens of treated mice were statistically higher than those of the control group. Kruskal-Wallis nonparametric test and Dunn multiple comparison. b) Measurement of the population of T CD8 + cells, the graph shows the percentages of this population.

Figure 5.

The mutein is more efficient than native IL-2 in the reduction of metastases in the experimental metastasis model of melanoma MB16F0 line. a) Representative photographs of lungs for each treatment. b) Quantification of pulmonary metastases in each group.

Figure 6. The treatment with the combination of the mutein and the OVA/VSSP vaccine potentiate the antitumor effect of the vaccine. The tumor-bearing mice were treated with the OVA/VSSP vaccine alone or in combination with the mutein. The graph shows the tumor growth curves, the group treated with the combination showed greater tumor growth reduction, being statistically different form the control group.

EXAMPLES

Example 1. Design of the IL-2 muteins

The muteins were designed computationally, from bioinformatics techniques, using as basis the reported structure of human IL-2 in database PDB (Protein Data Bank) and the amino acid sequences of IL-2 in various species that are available in Swissprot

database. Several muteins were designed including 3 to 6 mutations (introducing non-conservative amino acid substitutions) in solvent-exposed and highly conserved residues. These muteins were expressed in *E.coli* from a plasmid construction in pET28a vector including a target sequence of 6 histidines at the amino terminal. The muteins were purified by reverse phase (Figure 1) and a high purity (> 95%) was obtained. The muteins obtained were selected from their properties in experimental assays both in vitro and in vivo, to show the 3 basic properties described in the body of this invention. Out of all the muteins constructed Table 1 describes a set of specific mutations that have the desired property of being agonists of the activity of IL-2 without appreciably stimulating regulatory T cells and which show greater therapeutic efficacy than native IL-2 in the treatment of transplantable murine tumors. Table 2 shows the others muteins constructed that did not show the desired properties.

Table 2: Muteins constructed that do not have the basic properties described in this patent. The mutations are referred to according the numbering of the human IL-2.

Mutations
Q22V, Q126A, I129D, S130G
L18N, Q126Y, S130R
Q13Y, Q126Y, I129D, S130R
L18N, Q22V, T123A, I129D, S130R
R38A, F42A, Q126Y, I129D
Q126Y, I129D, E62L, E68V

Example 2. Demonstration of the agonist nature of the IL-2 muteins designed.

Figure 2 illustrates how the muteins mentioned in Table 1 bind to the components of the IL-2 receptor on the surface of CTLL2 cell line (Figura.2a). The constructed muteins bind to the CTLL2 cells, which are known to have on their surface both high affinity and intermediate affinity receptors for IL-2. The binding detected in our assays appears to be similar to that obtained with native IL-2. Figure 2b illustrates the capacity of the muteins shown in Table 1 to stimulate the growth of CTLL2 cell line (FIG. 2b). These muteins behave as partial agonists of the activity of IL-2 in this assay. Their specific activity is between 5 and 50 times lower than that of native IL-2.

Example 3. Effect of IL-2 muteins on regulatory T cells.

The muteins described in Table 1 show a very low capacity to stimulate regulatory T cells in vitro (Figure 3). As shown in this figure, while the native IL-2 is able to induce strong proliferation of regulatory T cells (T CD4+CD25+FoxP3+) stimulated with plate-bound anti-CD3 antibody. The muteins described in Table 1 in mass concentrations significantly higher than that of the native IL-2 did not stimulate the regulatory T cells. It must be added that the results described above are valid even if the amount of mutein to use is increased so as to use an amount equivalent in activity to the native IL-2 in the proliferation assay with CTLL2 line. The muteins described in Table 1 typically exhibit a capacity to stimulate regulatory T cells at least 1000 times lower than that of native IL-2

Example 4. Characterization of the *in vivo* immunostimulatory activity of the muteins designed.

The muteins described in Table 1 show an increased *in vivo* immunostimulatory capacity. Figures 4 a, b show how the muteins induce a splenomegaly greater than that of the native IL2 in naive mice, after treatment for five days with two daily doses of 20 µg of the mutein administered intraperitoneally. This stimulation correlates with a clear increase of effector populations, like T CD8+ lymphocytes. As relevant observation we can state that treatment with these muteins does not stimulate expansion of regulatory T cells (T CD4+CD25+FoxP3+) in contrast to what was observed in the case of the native IL2 (Fig. 4c, d).

Example 5. Measuring the therapeutic efficacy of the muteins in a murine model of transplantable tumors.

The increase in the therapeutic efficacy of the muteins designed in a murine model of transplantable tumors was proven. The muteins described in Table 1 show an increased efficacy for the treatment of lung metastases in a MB16 murine melanoma model. Figure 5 shows how treatment for 5 days with two daily doses of 20µg of the muteins in Table 1 administered intraperitoneally has a strong antimetastatic effect, which is not observed in the groups treated with equal doses of native IL-2.

Example 6. Measurement of mutein capacity for potentiating the antitumor vaccine effect

The capacity of designed mutein, for potentiating the antitumor vaccine effect was proven. It was used a primary tumor model with the EG7 cell line, a tumor cell line

genetically modified for expressing OVA antigen. The tumor-bearing mice were immunized with OVA antigen adjuvanted with VSSP alone, or in combination with the mutein. Figure 6 shows that the reduction of tumor growth was greater for mice treated with the combination, than for mice treated with the vaccine alone.

5

CLAIMS

1. An agonist polypeptide of IL-2, characterized by 95% homology with the sequence of the native IL-2 and wherein the said polypeptide is at least 1000 times less effective in stimulating *in vitro* and/or *in vivo* regulatory T cells and shows greater *in vivo* therapeutic efficacy.
2. The polypeptide of claim 1 which comprises mutations R38K, F42I, Y45N, E62L, E68V.
3. The polypeptide of claim 1 which comprises mutations R38K, F42Q, Y45E, E68V.
4. The polypeptide of claim 1 which comprises mutations R38A, F42I, Y45N, E62L, E68V.
5. The polypeptide of claim 1 which comprises mutations R38K, F42k, Y45R, E62L, E68V.
6. The polypeptide of claim 1 which comprises mutations R38K, F42Q, Y45E, E68V.
7. The polypeptide of claim 1 which comprises mutations R38A, F42A, Y45A, E62A.
8. A fusion protein comprising the immunomodulatory polypeptide of any of the claims from 1 to 7, coupled to a carrier protein.
9. The fusion protein of claim 8 wherein the carrier protein is Albumin.
10. The fusion protein of claim 8 wherein the carrier protein is the Fc region of human immunoglobulins.
11. A pharmaceutical composition useful in the treatment of cancer and chronic infectious diseases, characterized by comprising as active ingredient the polypeptide of any of the claims from 1 to 7.
12. The pharmaceutical composition of claim 11, characterized by comprising as active ingredient one or several polypeptides described in claims from 1 to 7.
13. A pharmaceutical composition useful in the treatment of cancer and chronic infectious diseases, which comprises as active principle the fusion protein described in any one of claims from 8 to 10.
14. Use of any of the polypeptides from claims 1 to 7 for the direct treatment of malignant tumors in replacement of native IL-2.
15. Use of any of the polypeptides from claims 1 to 7 to enhance the cellular and/or humoral response to vaccines used to replace native IL2.
16. Use according to claim 15 when the vaccine to promote is a therapeutic vaccine for cancer.

17. Use according to claim 15 when the vaccine to enhance is a vaccine for infectious diseases where regulatory T cells are relevant.
18. The polypeptide of any of the claims from 1 to 7 for the manufacture of a medicament useful in the treatment of chronic diseases.
19. The polypeptide of any of claims from 1 to 7 for the manufacture of a medicament useful in cancer therapy.
20. The polypeptide of any of the claims from 1 to 7 for the manufacture of a medicament useful in the therapy of chronic infectious diseases.
21. Use of the polypeptide of any of the claims from 1 to 11 for the therapy of chronic diseases.
22. Use of the polypeptide of any of the claims from 1 to 11 for cancer therapy.
23. Use of the polypeptide of any of the claims from 1 to 11 for the therapy of chronic infectious diseases.
24. Use of the fusion protein of any of the claims from 8 to 10 for the therapy of chronic diseases.
25. Use of the fusion protein of any one of the claims from 8 to 10 for cancer therapy.
26. Use of the fusion protein of any one of claims from 8 to 10 for the therapy of chronic diseases.
27. The polypeptide of any of the claims from 1 to 7 for the manufacture of a drug that modulates the immune system.
28. The polypeptide of any one of the claims from 1 to 7 and their applications when new mutations that increase their binding affinity to the different components of IL-2 are introduced.

SEQUENCE LISTING

<110> Centro de Inmunología Molecular

<120> Il-2 derivatives polypeptides with agonist activity for therapy of cancer and chronic infections.

<130> 402010CU00

<140> CU/P/2010/216
<141> 2010-11-12

<160> 6

<170> PatentIn version 3.5

<210> 1
<211> 133
<212> PRT
<213> E. coli

<400> 1

Ala Pro Thr Ser Ser Ser Thr Lys Lys Thr Gln Leu Gln Leu Glu His
1 5 10 15

Leu Leu Leu Asp Leu Gln Met Ile Leu Asn Gly Ile Asn Asn Tyr Lys
20 25 30

Asn Pro Lys Leu Thr Lys Met Leu Thr Ile Lys Phe Asn Met Pro Lys
35 40 45

Lys Ala Thr Glu Leu Lys His Leu Gln Cys Leu Glu Glu Leu Leu Lys
50 55 60

Pro Leu Glu Val Val Leu Asn Leu Ala Gln Ser Lys Asn
Phe His Leu
65 70 75
80

Arg Pro Arg Asp Leu Ile Ser Asn Ile Asn Val Ile Val
Leu Glu Leu
85 90
95

Lys Gly Ser Glu Thr Thr Phe Met Cys Glu Tyr Ala Asp
Glu Thr Ala
100 105
110

Thr Ile Val Glu Phe Leu Asn Arg Trp Ile Thr Phe Ser
Gln Ser Ile
115 120 125

Ile Ser Thr Leu Thr
130

<210> 2
<211> 133
<212> PRT
<213> E. coli

<400> 2

Ala Pro Thr Ser Ser Ser Thr Lys Lys Thr Gln Leu Gln
Leu Glu His
1 5 10
15

Leu Leu Leu Asp Leu Gln Met Ile Leu Asn Gly Ile Asn
Asn Tyr Lys
20 25

30

Asn Pro Lys Leu Thr Lys Met Leu Thr Gln Lys Phe Glu
Met Pro Lys
35 40 45

Lys Ala Thr Glu Leu Lys His Leu Gln Cys Leu Glu Glu
Glu Leu Lys
50 55 60

Pro Leu Glu Val Val Leu Asn Leu Ala Gln Ser Lys Asn
Phe His Leu
65 70 75
80

Arg Pro Arg Asp Leu Ile Ser Asn Ile Asn Val Ile Val
Leu Glu Leu
85 90
95

Lys Gly Ser Glu Thr Thr Phe Met Cys Glu Tyr Ala Asp
Glu Thr Ala
100 105
110

Thr Ile Val Glu Phe Leu Asn Arg Trp Ile Thr Phe Ser
Gln Ser Ile
115 120 125

Ile Ser Thr Leu Thr
130

<210> 3
<211> 133
<212> PRT

muteínas_ST25

<213> E. coli

<400> 3

Ala Pro Thr Ser Ser Ser Thr Lys Lys Thr Gln Leu Gln Leu Glu His
1 5 10 15

Leu Leu Leu Asp Leu Gln Met Ile Leu Asn Gly Ile Asn Asn Tyr Lys
20 25 30

Asn Pro Lys Leu Thr Ala Met Leu Thr Ile Lys Phe Asn Met Pro Lys
35 40 45

Lys Ala Thr Glu Leu Lys His Leu Gln Cys Leu Glu Glu Leu Leu Lys
50 55 60

Pro Leu Glu Val Val Leu Asn Leu Ala Gln Ser Lys Asn Phe His Leu
65 70 75 80

Arg Pro Arg Asp Leu Ile Ser Asn Ile Asn Val Ile Val Leu Glu Leu
85 90 95

Lys Gly Ser Glu Thr Thr Phe Met Cys Glu Tyr Ala Asp Glu Thr Ala
100 105 110

Thr Ile Val Glu Phe Leu Asn Arg Trp Ile Thr Phe Ser Gln Ser Ile
115 120 125

Ile Ser Thr Leu Thr
130

<210> 4
<211> 133
<212> PRT
<213> E. coli

<400> 4

Ala Pro Thr Ser Ser Ser Thr Lys Lys Thr Gln Leu Gln Leu Glu His
1 5 10 15

Leu Leu Leu Asp Leu Gln Met Ile Leu Asn Gly Ile Asn Asn Tyr Lys
20 25 30

Asn Pro Lys Leu Thr Lys Met Leu Thr Lys Lys Phe Arg Met Pro Lys
35 40 45

Lys Ala Thr Glu Leu Lys His Leu Gln Cys Leu Glu Glu Leu Leu Lys
50 55 60

Pro Leu Glu Val Val Leu Asn Leu Ala Gln Ser Lys Asn Phe His Leu
65 70 75 80

Arg Pro Arg Asp Leu Ile Ser Asn Ile Asn Val Ile Val
Leu Glu Leu
85 90
95

Lys Gly Ser Glu Thr Thr Phe Met Cys Glu Tyr Ala Asp
Glu Thr Ala
100 105
110

Thr Ile Val Glu Phe Leu Asn Arg Trp Ile Thr Phe Ser
Gln Ser Ile
115 120 125

Ile Ser Thr Leu Thr
130

<210> 5
<211> 133
<212> PRT
<213> E. coli

<400> 5

Ala Pro Thr Ser Ser Ser Thr Lys Lys Thr Gln Leu Gln
Leu Glu His
1 5 10
15

Leu Leu Leu Asp Leu Gln Met Ile Leu Asn Gly Ile Asn
Asn Tyr Lys
20 25
30

Asn Pro Lys Leu Thr Lys Met Leu Thr Ile Lys Phe Glu
Met Pro Lys
35 40 45

Lys Ala Thr Glu Leu Lys His Leu Gln Cys Leu Glu Glu
Glu Leu Lys
50 55 60

Pro Leu Glu Val Val Leu Asn Leu Ala Gln Ser Lys Asn
Phe His Leu
65 70 75
80

Arg Pro Arg Asp Leu Ile Ser Asn Ile Asn Val Ile Val
Leu Glu Leu
85 90
95

Lys Gly Ser Glu Thr Thr Phe Met Cys Glu Tyr Ala Asp
Glu Thr Ala
100 105
110

Thr Ile Val Glu Phe Leu Asn Arg Trp Ile Thr Phe Ser
Gln Ser Ile
115 120 125

Ile Ser Thr Leu Thr
130

<210> 6
<211> 133
<212> PRT
<213> E. coli

<400> 6

Ala Pro Thr Ser Ser Ser Thr Lys Lys Thr Gln Leu Gln
Leu Glu His
1 5 10

15

Leu Leu Leu Asp Leu Gln Met Ile Leu Asn Gly Ile Asn Asn Tyr Lys
20 25 30

Asn Pro Lys Leu Thr Ala Met Leu Thr Ala Lys Phe Ala Met Pro Lys
35 40 45

Lys Ala Thr Glu Leu Lys His Leu Gln Cys Leu Glu Glu Ala Leu Lys
50 55 60

Pro Leu Glu Glu Val Leu Asn Leu Ala Gln Ser Lys Asn Phe His Leu
65 70 75 80

Arg Pro Arg Asp Leu Ile Ser Asn Ile Asn Val Ile Val Leu Glu Leu
85 90 95

Lys Gly Ser Glu Thr Thr Phe Met Cys Glu Tyr Ala Asp Glu Thr Ala
100 105 110

Thr Ile Val Glu Phe Leu Asn Arg Trp Ile Thr Phe Ser Gln Ser Ile
115 120 125

Ile Ser Thr Leu Thr

130