A computational simulation of the interaction between immune and neuroendocrine systems

R. Pizzi, T. Rutigliano, P. Guadalupi and M. Pregnolato

Abstract - Interesting hints of a neuroendocrine–immune system cross-talk at several biological levels have been brought by many research papers during the last decades, although no scientific evidence has been fully established. In this study we hypothesize that efficient neuroendocrine-immune systems interactions may be identified at the membrane receptor level, and could be highlighted by a structural bioinformatics research. In this paper we built a model of the interaction between a typical gastrointestinal cancer membrane with several substances that are supposed to be involved in the immune response. A computational docking analysis shows that the interaction between melatonin, as a neuroendocrine agent, and other immune substances and mediators of the inflammatory response may have a role in the complex relationship between nervous and immune system.

Keywords – bioinformatics, docking, immune system, Interleukin, IL-2, melatonin, LPS, cancer, PNEI, placebo.

I. INTRODUCTION

Since from the 1980s scientific evidences have been brought that hormones and cytokines are involved in a functionally relevant cross-talk between CNS and the immune system. It was shown that immune response, among other effects, alters the activity of hypothalamic noradrenergic neurones [1,2,3] .

The main hypothesis set for neuroimmune interaction is that fluctuations in neuroendocrine function should result in immune changes, and conversely, fluctuations in immune activity should result in neuroendocrine changes [4].

It can be hypothesized that neural alterations following immune activation may be mediated by chemical messengers produced by activated cells of the immune system, and neuroendocrine signals may feedback to alter immune function after immunological activation.

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For this purpose, immunological cells may express receptors for hormones and neurotransmitters.

An interesting neuroendocrine substance is for example melatonin.

Melatonin (MLT) is a hormone secreted by the pineal gland in the brain; it helps regulate other hormones and maintains the body's circadian rhythm. Melatonin also helps control the timing and release of female reproductive hormones. Melatonin is reported to have strong antioxidant anti-aging effects, and preliminary evidence suggests that it may help strengthen the immune system [5,6] and can be considered a co-stimulator able to activate the T cells and stimulate the cytokines production [7,8,9,10,11,12,13,14]. Melatonin is also claimed to exert both direct and indirect anticancer effects in factorial synergy with other molecules [15].

In particular, Lissoni et al. [16] carried out a protocol aimed to artificially build an antitumor immunity, administering patients MLT, low doses of interleukine 2 (IL-2), cytokine responsible for a specific immune response. They observed a 20% of tumor regression in patients unsuitable for standard therapies, reaching a 3 years survival in around 10% of patients.

Furthermore, Lissoni reported that the use of MLT is useful to reduce chemotherapy toxicity.

It has been shown [17] that MLT secretion in neoplastic patients reduces itself sharply and disappears at the terminal stage; post-mortem analyses show a vacuolization of the pineal gland.

However, the scientific evidence of the therapeutic efficacy of substances claimed to be active by the psychoneuroimmunology (PNEI) researchers [18,19,20] is not well established yet.

Similarly, another interesting phenomenon studied by PNEI that bounds immune and nervous system but still lacks of a complete explanation of its physiological mechanism is the placebo effect, that constitutes an endogenous therapeutic response [21,22].

As well-known, placebo effect is a phenomenon in which a placebo - a fake treatment, an inactive substance like sugar, distilled water, or saline solution - can sometimes improve the patient's conditions simply because the person has the expectation that it will be helpful.

Since the publication of Henry K. Beecher's in 1955 [23], the phenomenon has been considered to have clinically important effects.

The evidence of the effect is such that, to separate it from a drug's true medical benefits, companies seeking governmental approval of a new treatment use placebo-controlled drug studies.

There is accumulating evidence from different methodological approaches that the placebo effect is a neurobiological phenomenon [24,25,26,28,29].

Evoked brain potentials, PET, functional imaging showsthat placebo links to the activation of many brain areas [30,31,32,33,34,35,36].

It has been shown that placebo analgesia depends upon the release in the brain of endogenous opioids since 1978 [37] . Dopaminergic pathways may underlie these responses [38], and a serotonin-related pathway has been also hypothesized [39].

Finally, a recent work claims the placebo effect is due to an immunomodulatory control by the brain [40].

Our working assumption is that the efficacy of the interaction between active substances of the neuroendocrine and immune system may be due to a reciprocal synergy at a membrane receptors level [41,42], that could be highlighted by means of a structural bionformatics study.

For this purpose we developed a model of the interaction between the mentioned molecules IL-2, MLT and a third one, the Lipopolysaccharide (LPS), an antigen of Gram-negative bacteria, involved in inflammation and infection.

IL-2 is necessary for the growth, proliferation, and differentiation of thymic-derived lymphocytes (T cells) to become 'effector' T cells. IL-2 is normally produced by T cells during an immune response [43,44] and it is used at an experimental level in several therapies in oncology [45,46,47,48,49,50].

LPS raises the level of inflammation and attracts immune substances; in presence of infection a specific immune response occurs, directed to antigens and/or antibody epitopes. The overstimulation of the immune system caused by LPS was proposed to be useful in oncology, and LPS is currently used in several antitumor therapies [51,52,53,54,55,56,57,58,59,60].

Therefore we considered to add LPS in our model, in order to observe the interation between a substance active at a neuroendocrine level (MLT), an element of the immune system (IL-2) and a stimulant of the immune system that is normally present in the human body (LPS).

II. Materials and Methods

A *The receptor-ligand complex*

The structure and functions of living cells are critically dependent on the formation and termination of associations between an impressive number of biomolecules, whose specific interactions regulate mechanical and topological properties [61, 62]. A cascade of activation of molecules will finally lead to bind to specific receptors scattered through the cells[63].

Most biological functions are mediated by interactions between ligands and proteins. The protein can interact with other proteins, with nucleic acids, with small ligands (e.g. metabolites or ions), and with more ligands simultaneously.

The interaction with a ligand can induce conformational changes that influences the activity or accessibility of other binding domains (substrate, protein, DNA) [64].

The molecules interact in a highly specific manner: the protein-ligand interaction is dictated primarily by electromagnetic and steric complementarity of the two compounds (i.e. the shape of the ligand is mirrored by the shape of the binding site) [65,66]. The first important parameter for protein-ligand complexes is therefore the interaction area.

The computational simulation techniques allow an extended exploration of the interaction between realistic models of macromolecules [67].

In this work a molecular docking computational simulation has been adopted.

B *The docking procedure*

We aimed to analyse the interactions between the mentioned structures taking into account their known receptors. Using a docking procedure we could study *in silico* conformational changes starting from the involved attractive forces, considering molecules both individually and in combination. The application of molecular docking methods aims to predict the strength of association or the binding affinity between two molecules, and the orientation of small molecules binding to a protein target. This methodology is extremely useful when structural information (obtained e.g. by Nuclear Magnetic Resonance (NMR) or Circular Dichroism (CD) or X-rays) are not available for the involved intermolecular complex and cannot be found in the Protein Data Bank (PDB) [68]. Docking methods are also applied for the study of the energy and geometry binding properties of potential new drugs, highlighting new binding modes of already known drugs, or searching for molecules that could potentially become active to new receptor targets (virtual screening), realizing an "*in silico*" forecast of the pharmacological activity of new molecules.

As we have seen, the purpose of an automatic molecular docking algorithm is to develop methods capable to predict the geometry of binding through a score function that estimates the affinity between target and ligand. Different types of score functions have been implemented: force field based, knowledge based, consensus scoring etc.[69,70,71]

The main computational problem is that, in the process of molecular docking, a large number of conformational degrees of freedom must be taken into account. Several algorithms have been developed for this purpose.

If the bond angles, bond lengths and torsion angles of the components are not modified at any stage of the procedure, we speak of rigid body docking. Docking procedures which permit conformational changes, or flexible docking procedures, are computationally expensive and they must face the complex task to select a small subset of possible conformational changes.

The rigid docking procedure considers the two interacting structures as rigid, taking into account only six translational and rotational degrees of freedom of the ligand with respect to the bigger molecule, that is considered fixed.

In this approach the choice of the ligand conformation is crucial, as it must correctly approach the other molecule in the intermolecular complex.

Most of the molecular docking algorithms generate a large number of possible structures, which must then be evaluated in order to select for subsequent analysis a smaller, but representative set of conformations that could be the most likely similar to the real docking mode.

This is often realized using cluster analysis. Belonging to a cluster depends on how much the element under consideration is far from the cluster or close to it. When comparing different conformations, the most commonly used measure is the RMSD (root mean square distance) between pairs of atoms.

In our study the docking conformations and interaction energies were performed using the HEX docking system [72] , that allows both calculation and 3D visualization with GPU management to accelerate processing.. The ligand finds its position into the protein's active site after a certain number of movements in the conformational space. The interaction between molecules takes place on the basis of their 3D shape and of their electrostatic complementarity.

HEX uses a rigid docking procedure: assuming that protein and ligand are rigid structures, it performs a spatial matching of the geometrical characteristics of protein and ligand. In rigid docking the receptor molecule is considered fixed on the three-dimensional space and all the possible positions and orientations of the ligand in space are evaluated , including internal changes of the ligand structure by torsion angle rotations.

The search procedure must take into account the six degrees of freedom: three translations and three rotations. The first computationally efficient algorithm to determine the geometric complementarity between two molecular structures, able to solve the problem of rigid docking, was presented by Katchalski-Katzir et al. in 1992 [73]. This method consists of an automatic procedure that projects the molecule in a 3D grid, performing a distinction between surface and interior atoms. Then it calculates, using the Fourier transform, a correlation function that evaluates the overlapping degree of the molecular penetration relative to all the possible orientations of the molecule ligand [74]

HEX gets further and uses a FFT evolution called SPF (Spherical Polar Fourier). Each molecule is modelled in three dimensions using parametric functions that encode also the surface spatial potential distribution and are based on the expansion of spherical orthogonal functions. The correlation (or overlap as a function of translation/rotation operations) between a pair of 3D functions can be calculated using expressions which are similar to the conventional FFT docking methods.

This new approach allows to analyze in detail and quickly all the global features of a macromolecule protein, representing it with a surface formed by spheres. The spheres represent both the spatial surface and the distribution of the potential, and through the research of complementarity of these surfaces, followed by a further energy minimization of the complex, it is possible to define the possible surfaces of interaction.

A series of solutions is generated and a clustering procedure classifies all the possible solutions with a scoring system based on a RMSD minimization, as explained above, ordering them in such a way that the first solution is the most likely to be similar to the real biological docking.

Through this new approach it is possible to analyze in detail and quickly all the global features of a macromolecular protein.

C Experimental Design

Fundamental antitumor immunity cells are the CD4+ type 1 lymphocytes and the dendritic cells. The CD4 + lymphocyte induces the production of IL-2 cytokine with several biological actions, such as T and B lymphocyte proliferation and activation of natural killer cells (NK). As mentioned above, our analysis is not limited to study the IL-2 and MLT cocktail, because our interest is also devoted to a third molecule (LPS), antigen of Gram-negative bacteria. IL-2 (PDB code: 1M47), MLT (PDB code: DB01065) and LPS (PDB code: 3GLV), and their known receptors: IL-2R (PDB code: 2ERJ), MT1 (PDB code: 1L9H), and Toll-like receptor 4 (TLR4) (PDB code: 3FXI) respectively, have been anchored to a lipid membrane with typical features of gastrointestinal cancer, as specified below.

We aimed to emulate a membrane with the features of adenocarcinoma, a malignant and undifferentiated epithelial tissue, which originates from glandular epithelium. For this purpose we selected a portion of the Palmitoyloleoylphosphatidylcholine membrane (POPE), a large bilayer with 340 lipids [75,76].

In histology, a signet-ring cell is a malignant cell type seen predominantly in carcinomas [77]. In these cells nucleus is pushed to periphery, villin (VIL, PDB code: 2K6N), ezrin (EZR, PDB code: 1NI2) and fimbrin (FIM, PDB code: 1AOA) proteins are localized on membrane surface forming finger-like structures (microvilli), made up of a membrane element and a lining cytoskeletal structure. Villin is the best characterized protein [78].

First of all we carried out the *in silico* anchoring of VIL, EZR and FIM, secondly we introduced cellular receptors IL-2R, MT1 and TLR4, and only as a last step, we performed molecular docking with the ligands IL-2, MLT and LPS.

II. Results

As previously reported, we realized the docking procedure at successive stages: first we anchored the receptors to the membrane, then we carried out the docking between ligands and receptors. The ligands were introduced one at a time allowing to see the binding sites involved and the conformational variations.

A visual analysis of the resulted interactions was performed using Swiss-PdbViewer 4.1.0 [79].

Colours can be appreciated in the online version.

A IL-2

The IL-2 ligand (3-D shaped, in the lower left quadrant, light blue), in configuration with all receptors, is positioned on the opposite surface with respect to the IL-2 receptor (transmembrane, yellow). The PDB configuration of this receptor shows other IL-2 molecules (upper right, orange). The IL-2 ligand is positioned in a different area, in proximity of MT1 (lower right, blue), and it is far from IL-2 molecules incorporated into receptor itself (Fig.1).

The IL-2 ligand in presence of MLT (small, immersed in the upper side of the membrane, 3-D shaped, fuchsia) changes completely its position, by moving to the surface where the docking with IL-2 receptor becomes predominant, overlapping, in addition, another IL-2 molecules present in the model (Fig.2).

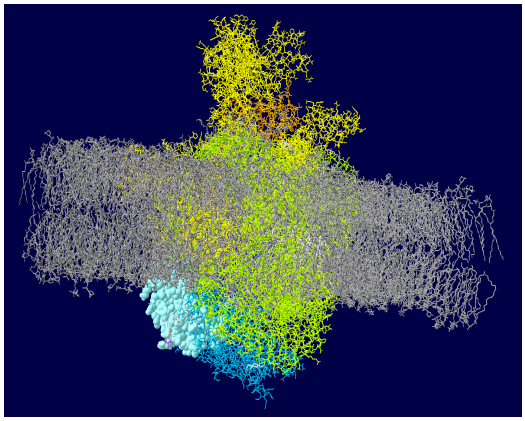

Fig.1 IL-2

The IL-2 ligand (3-D shaped, light blue), in configuration with all receptors, is positioned on the opposite surface with respect to the IL-2 receptor (transmembrane, yellow).

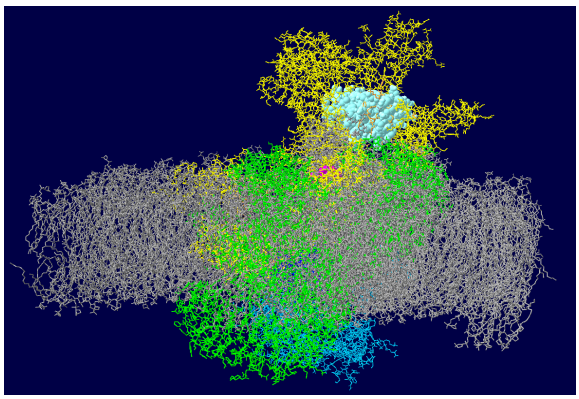

Fig.2 IL-2 and MLT

The IL-2 ligand in simultaneous presence of MLT (immersed in the upper side of the membrane, 3-D shaped, fuchsia) changes completely its position moving to the upper bilayer surface. In this conformation the IL-2 ligand binds IL-2R and seems to be more compacted. In this conformation the IL-2 ligand under study is superposed on the IL-2 ligand already included in the IL-2R PDB (the same situation will be observed in configuration with three ligands, Fig. 4).The MLT position seems to be not influenced by the presence of the IL-2 ligand.

B MLT

The MLT ligand is positioned away from the MT1 receptor (lower right, blue) in all the configurations. MLT partially varies its position from the configuration where it is the only ligand (Fig. 5) with respect to the configuration with IL-2 (Fig. 2). MLT in the presence of LPS (3-D shaped, on the right, Fig.6) seems to penetrate deeper into the membrane. The MLT position seems to be not influenced by the simultaneous presence of the IL-2 ligand and LPS: the configuration obtained for this ligand is the same of Fig. 2 .

C LPS

As a single ligand (Fig.7) and / or in the presence of the other ligand (Fig.3 and Fig.6), LPS is positioned transversely in the membrane, passing through it completely. Inside the TLR4 complex (lower right quadrant, green) there is a portion of LPS (lower right quadrant, dark blue, to the left of TLR4), and the presence of this portion of LPS does not seem to affect any of the examined molecules (perhaps because it represents just a small portion). The LPS ligand is not superimposed to any of these molecules in any configuration, but the LPS location is completely distorted in the combination with the three ligands (Fig 4), where it compresses itself, localizing in proximity to the upper surface, in correspondence with the IL-2 receptor and making contacts with the IL-2 ligand.

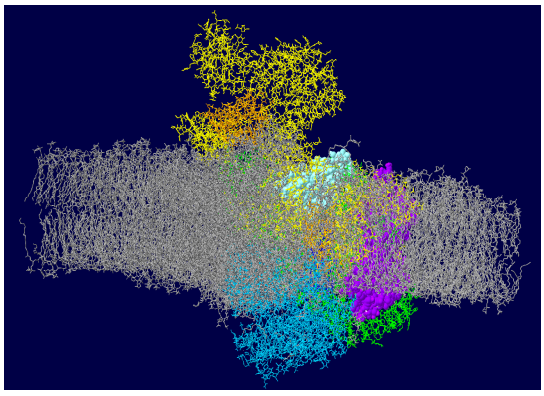

Fig.3 IL-2 and LPS

The IL-2 ligand (left 3-D shaped, light blue) in presence of the LPS ligand (right 3D-shaped, purple) moves again: it is now embedded into the membrane, in contact with the

transmembrane portion of IL-2R and with the TLR4 complex (lower right quadrant, green). The LPS position is not so different compared to that in Fig.7 (unique ligand).

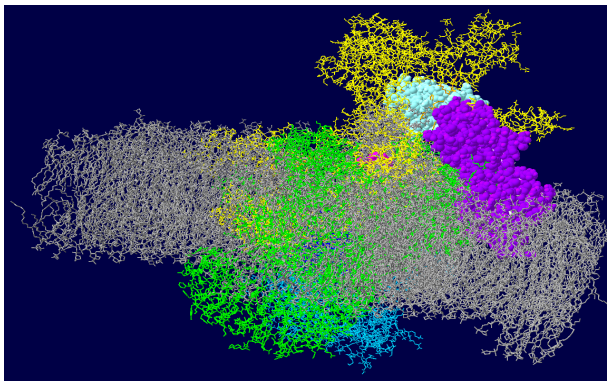

Fig.4 IL-2 and MLT and LPS

The IL-2 ligand (3-D shaped on the left, light blue) in simultaneous presence of MLT (3-D shaped centered inside the membrane, fuchsia) and LPS (3-D shaped on the right, purple) restores the same position obtained in configuration with MLT (Fig. 2). IL-2 shows also an important contact area with the LPS ligand .The MLT position seems to be not influenced by the simultaneous presence of the IL-2 ligand and LPS: the configuration obtained for this ligand is the same as in Fig. 2. LPS changes completely its position moving from a transmembrane to a superficial one. It is close to the IL-2 ligand and to IL-2R (transmembrane, upper right, yellow).

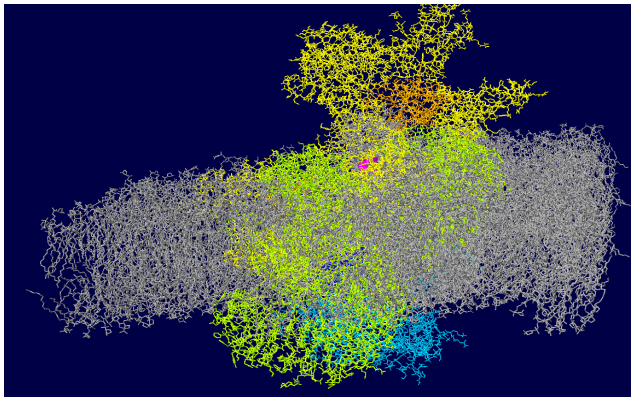

Fig.5 MLT

The MLT ligand ((3-D shaped centered inside the membrane, fuchsia) is far from its known receptor MT1 (lower right, blue) on the bottom. Instead, MLT is positioned in the area of IL-2R (transmembrane, yellow)

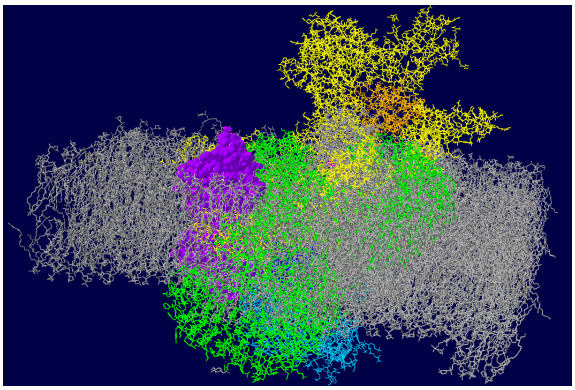

Fig. 6 MLT and LPS

In presence of the LPS ligand (3-D shaped, purple), MLT has the same position of Fig. 5 but it is more embedded into the membrane.The LPS position is not so different from the case in Fig. 7, where it is the only ligand.

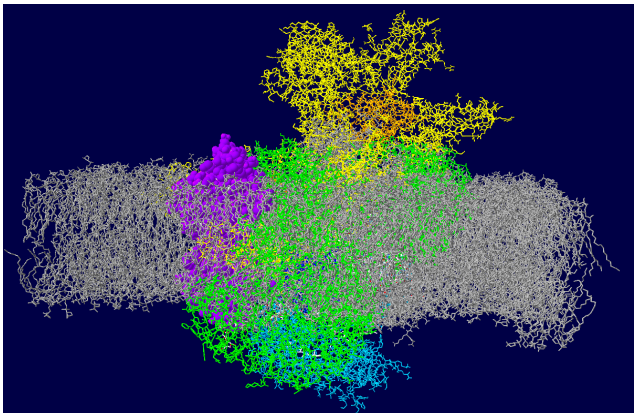

Fig.7 LPS

The LPS ligand is positioned in the TLR4 complex (transmembrane, green), but also close to an inner IL-2R area (upper right, yellow). It takes a transmembrane position. It is not superposed to the portion of LPS already included into the TRL4 complex (lower right, blue).

IV. Conclusions

The reported research was carried out using a computational simulation based on a docking procedure. The docking

analysis performed in succession: we added one molecule at a time because we were interested in observing intermediate conformations as well. The docking results showed indeed some interesting features.

We were able to verify and confirm that IL-2 binds its specific receptor (IL-2 receptor) and LPS binds TLR4, as already known in the literature. This result could also be used as an internal control to validate our computational method. We also have found that some ligands, and their receptors, behave in a different way when other structures are present. The behaviour of all the ligands is significantly different in the presence of all the three molecules.

- IL-2: in our model three molecules of IL-2 are present. The receptor of IL-2 is a heterotrimeric molecule present in the complete form only on lymphocytes activated by the antigen. In normal conditions, only two subunits are present, and this complex shows a low affinity for the ligand, which must be very concentrated in order to bind: hence the reason why the two molecules of IL-2 appear already present in the receptor. By adding a third molecule to the system that presents already two incorporated molecules in the receptor, namely the IL-2 ligand actually used for the simulations, we noted that it does not bind the designated receptor. By adding this third IL-2 molecule plus at least one other ligand (LPS or MLT), new positions and new conformations are reached. It is shown that IL-2 needs the presence of at least one other ligand to dock the IL-2 receptor area. The model configurations "LPS + IL-2" and "IL-2 + MLT + LPS" show conformational changes that seem related to the presence of the other molecules and do not seem to be related to the IL-2 concentration. IL-2 is embedded into the membrane only in presence of LPS: it seems that IL-2 is able to attract LPS or vice versa.

- MLT: melatonin is not located exclusively in its MT1 receptor, but it is positioned in the proximity of the IL-2 receptor even though it does not make contact with it. IL-2R reveals to be an important receptor also for the MLT ligand. LPS is able to drag MLT into the membrane, and the IL-2 ligand does not affect the MLT position.

- LPS: the position is transmembrane in two out of three configurations. LPS position is distorted in presence of IL-2 and MLT at the same time obtaining a superficial position on membrane surface. The LPS presence is enough to change IL-2 configuration but not vice versa. A new conformational position is shown in presence of other molecules, and LPS is attracted by them where there are at least two. Probably the distortion of the configuration is not due to the attraction of one or the other molecule, but to the forces interacting in the field. Interestingly, LPS moves from a location inside the membrane to the surface, and perhaps the superficial position could result in the activation of receptors and thus in the induction of a further immune response: this hypothesis should be confirmed in subsequent studies.

In conclusion, this preliminary study has allowed to observe that the simultaneous presence of MLT, IL-2 and LPS resulted in changes of the ligands positions if compared to their positions inside the natural receptors, that they occupy in the absence of other ligands.

LPS seems to be the strongest agent able to induce conformational changes. The IL-2 receptor appears to be of fundamental importance for all the ligands present in the study. Probably the surface of this receptor presents numerous hot spots, i.e. surface protein regions that mostly contribute to the binding, enhancing its effect. It is well known that these hot spots tend to bind a variety of organic molecules [80].

It can therefore be concluded that what we had hypothesized, namely the existence of an interaction between neuroendocrine agents, immune substances and mediators of the inflammatory and/or antitumor response as LPS, has an effective confirmation at the level of computational simulation of a receptor-membrane system.

Subsequent studies can better shed light on the nature of this interaction and verify if the simultaneous presence of these three agents, or other agents belonging to the immune and neuroendicrine systems, may constitute an effective means to boost our immune response.

The computational simulation methods [81,82,83,84,85] can constitute a valid modeling tool that yields an initial assessment prior to the necessary *in-vitro* and *in-vivo* evaluations.

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