Immunotherapy and Immunoregulation: The Barrier of Complexity

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Early generalization and reductionism are professional diseases of scientists, especially in Biology. Cancer Immunotherapy is an excellent example: each time a new component of the system has been identified, there have been attempts to place it at the middle of explanations and to use it alone as a tool or as a target of therapeutic interventions. So it happened with tumor associated antigens, monoclonal antibodies, interferon, interleukin-2, Lak cells and others. And each time we have faced the same barriers: the redundancy of biological systems and the robustness of their control loops.

More than 40 years have elapsed since the seminal experiments describing tumor-associated rejection antigens. A search of the literature since just 1990 identifies almost 400 000 scientific articles in immunology and more than 50 000 in cancer immunology, and still we have few products for cancer immunotherapy, most of them for infrequent malignancies, and still with very limited impact in survival.

The recognition of this reality called for a deep review of the theoretical basis on which we were working in cancer immunology. This was the major aim of this series of Immunotherapy Workshops held at the Center of Molecular Immunology in Cuba since 1994.

The first theoretical transition was to abandon the analogy between cancer immunotherapy and the immune response to infectious diseases, and to make use of the probably more fertile analogy between immune responses to tumors and autoimmune diseases [1].

Such a shift had tremendous practical implications (Figure 1).

In fact the "autoimmune analogy" drove research through different paths, sometimes divergent.

Part of the outcomes of this theoretical shift will be seen in the Abstracts following this paper, presented at the 5th edition of the International Workshop "Immunotherapy for the New Century", held in Havana, in December 2002. They will describe the implications of dominant tolerance for cancer immunotherapy, the use of monoclonal antibodies and vaccines directed to fully "self" molecules, the regulatory properties of some monoclonal antibodies, and attempts to manipulate the signals of the innate immune system in order to intervene (therapeutically) in the Self\Nonself categorization procedure embedded in the system. They will also show an enlightening joint discussion of immunotherapy experiments by scientists interested in cancer and those interested in autoimmune diseases.

But this meeting will go a step beyond recognizing the experimental consequences of the first theoretical transition (the autoimmune paradigm of cancer immunotherapy): it will try to point out the next theoretical transition which will come from surpassing the reductionist approach of molecular biology and make contact with the models, concepts and paradigms of what is emerging under the name of "new science of complexity" [2].

This transition is coincident with (and partially driven by) the hot polemics around the publication of the human genome in 2001, whose main message is that things are not so simple as previously imagined.

Molecular Biology was set on the hypothesis (explicit or not) that phenotypic complexity was embedded in the genome; but now we know that we have less than 35 000 genes (mice have 26 000), 98% analogous to chimpanzees. We had the dogma of "one gene = one protein" and now we know that alternative splicing occurs in nearly 40% of human genes, multiplying the number of proteins. It was thought that the double helix structure of DNA was a guarantee of fidelity in the copy, but now we know that copy errors are much more frequent *in vitro* than they are *in vivo*, which means that the fidelity in DNA replication is more than a property of the DNA molecule itself, but a property of the complex mixture of enzymes and proteins which operates timely in the replication machinery of the cell. It was thought that the aminoacid sequence of proteins determined their spatial shapes but now the discovery of chaperones and prions comes to complicate the picture.

1. Lage A. Mining at the intersection between cancer research and autoimmunity research. Biotecnología Aplicada 2001; 18 (1):33-4.

2. Carlson JM, Doyle J. Complexity and robustness. Proc Nat Acad Sci USA 2002; February 19, 99(1):2538-45.

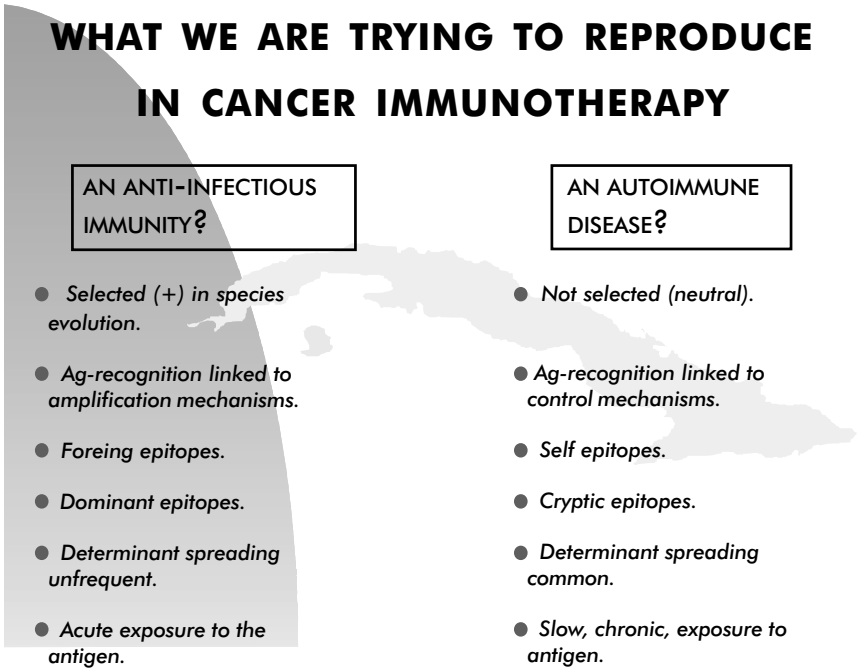

Figure 1. Basic mechanisms of immune protection against infections and of autoimmune disease are different. Understanding anti-tumor immunity as a kind of autoimmunity drivers research to different approaches.

Simultaneously, also Immunology started to accumulate evidences that things are not so simple, and many of them came from experiments with knock-out mice. Despite the central role of interleukin-2 in all charts of immune cells interplay, surprisingly IL-2 KO mice are normal with regard to thymocyte and peripheral T cell subset composition [3]. Something similar happened with β2-microglobulin KO-mice, which lack MHC-I and CD8 T-cells and have a normal development [4].

Examples like these are just remaining us that biological systems are tremendously redundant and degenerate (structurally different elements to perform the same function), full of non-linear interactions and amplification mechanisms (clonal expansion is exponential; signal transduction cascades are catalytical), many of them showing cooperativity, and producing surprising phase transitions and emerging properties. Sometimes there is a huge amplification of small changes (a small change of cell cycle time from 10 h to 8 h translate in 16 times increase in cell number over a 7 day experiment [5]); sometimes the system is robust to fluctuations in its components.

For the experimentalist what all this means is that there are severe limitations to the power of human intuition for the design and interpretation of experiments.

In fact, when mathematical modelling of complex systems started to be used, counterintuitive predictions often emerged, and some of them turned out to be true, as will be illustrated during this Workshop by presentations about the effect of immunosuppression on vaccinations [6] and with the prediction of proliferative activity of regulatory T-cells previously thought to be anergic [7].

Complex systems, composed by many components related to each other in networks of non-linear interactions, have an enormous amount of possible "states". Just a boolean network composed by 1000 nodes (imagine lamps) each one having the possibility of being "on or off" has $2^N = 2^{1000} = 10^{300}$ possible states and this figure is much bigger than the number of hydrogen atoms in the known universe. What to say then of a human immune system composed by 10^{12} lymphocytes in about 10^8 clones, each being either "naïve or activated"?

Fortunately real complex systems do not describe trajectories throughout all the space of states, but tend to be trapped by their own rules of interactions, into "attractors", conditions of the system in which it equilibrates or oscillates around. Moreover, these systems can be rather insensitive to random fluctuations or artificial modifications of some of their components, moving back to the same starting state. This behaviour is captured by the concept of "basin of attraction" [8].

Returning now to cancer immunology, when we think how many and diverse immunotherapy interventions (interferon, interleukin-2 with or without Lak cells, monoclonal antibodies, cancer vaccines) do achieve about 15-20% response rate in clinical trials (but rarely more than that) we could wonder if we are trapped in a "basin of attraction" from which we have not been able to move the system out, just by handling one of its components.

Can we do something?

Yes we can. Fortunately, the understanding of the huge complexity and robustness of biological systems need not to be paralyzing for Science. As very often happens the consciousness of a reality comes together with the tools to act on it. During the last decade we have witnessed the emergence of high throughput experimental technologies able to perform high amounts of simultaneous measurements of gene sequences, gene expression, protein maps, cell phenotypes and the like, together with the development of computer capacity and software to process, organize and explore this data flood. This handling of high volumes of quantitative information is not so different to what physical scientists have been doing for decades.

Simultaneously, modern theoretical biology based on the intensive use of computer-assisted mathematical modeling and simulation of biological systems, is increasingly providing tools for the interpretation of data, and for experimental design.

All this is what some people describe as the transition from Molecular Biology to System Biology [9].

As attempts to understand biology at the system level proliferate, another source of enlightening ideas could be the study of analogies between apparently different biological systems. Take for example the comparison between the organization and information handling procedures of the immune system and the central nervous system (CNS).

In an approach increasingly similar to the immune system, the CNS is currently viewed as an adaptive recognition system, based also in a random generation of a diverse repertoire (in this case the random establishment of connections among neurons) coupled to a process of somatic selection of connections with adaptive value [10]. Here again, we found an innate system for fast emotional responses, selected by the evolution of the species, but with a response repertoire of limited diversity, linked to a more evolutionary recent learning system, with an enormous diversity of responses, reflecting the adaptive experience of the individual.

Previous versions of the present Workshop had the merit of pooling together scientists working on cancer and scientists working on autoimmune diseases. Might be the forthcoming meetings will include neuroscientists as well.

Where are we going?

The critics of the extreme reductionism of molecular biology can not be translated into a critics of reductionism itself, because Science is intrinsically reductionist. Otherwise it would loose its explanatory and predictive capacities. The real question is how much reductionism we can take in order to keep our ability to predict and to modify a complex reality.

At the end of the journey there will always be the prediction and the experiment. But now they will be predictions and experiments of a different nature, attempting to capture directly a higher level of complexity.

There will be an explosion of "in silico experiments" simulating in the computer the effects of tumors, vaccines, immunosuppressive treatments and the like. We will see more cellular and animal experiments designed by the predictions of mathematical models and

3. Schorle H, Holtschke T, Hunig T, Schimpl A, Horak I. Development and function of T cells in mice rendered interleukin-2 deficient by gene targeting. Nature 1991; 352(6336):621-4.

4. Koller BH, Marrack P, Kappler JW, Smithies O. Normal development of mice deficient in beta 2M, MHC class I proteins, and CD8+ T cells. Science 1990;248 (4960):1227-30.

5. Germain RN. The art of the probable: system control in the adaptive immune system. Science 2001;293:240-5.

6. González G, Montero E, León K, Cohen IR, Lage A. Autoimmunization to epidermal growth factor, a component of the immunological homunculus. Autoimmunity Reviews 2002;1:89-95.

7. Oliveira V, León K, Caramalho I, Hori S, Haury M, Demengeot J, Carneiro J. Target CD4+CD25- T cells promote the growth of regulatory CD4+CD25+ T cells *in vitro*. J Immunology (submitted).

8. Kauffman S. At home in the universe: the search for laws of self organization and complexity. New York: Oxford University Press; 1995.

9. Kitano H. Systems biology: a brief overview. Science 2002;295:1662-4.

10. Edelman G.M. On the matter of mind. New York: Basic Books; 1992.

computer simulations. The intuition of the immunologist has a limited capacity to handle several variables and interactions simultaneously, and then in-silico simulations will come as a kind of "computer-assisted intuition".

Both mathematical models and real experiments will approach features of the immune system which have been mostly neglected "for the sake of simplicity", such as the organization of the system in real geographic compartments and virtual dynamic compartments for diverse cellular subpopulations.

There will be a shift in the interest from the study of cell clones to the study of the interactions among clones of different specificities (if we develop good tools to measure and to manipulate connectivity), a concept already anticipated by N. Jerne 30 years ago [11] but poorly operationalized up to now, an again analogous to the concept of neuron "maps" in brain sciences.

It can be predicted that many of these experiments will translate into a wider exploration, not necessarily empirical, of schedule-dependence in immunotherapy (doses, routes, intervals, sequences, etc), an approach which was tremendously fertile in cancer chemotherapy in the seventies.

Imagine a situation given by the availability of a monoclonal antibody and two cancer vaccines to the same target (as is currently happening at the Center of Molecular Immunology in its Epidermal Growth Factor targeted immunotherapy project) which could be combined with standard chemotherapy each one in three dose levels, using four different treatment sequences and just two different time intervals. An initial exploration of schedule dependence will demand 4 x 3 x 4 x 2 = 96 clinical trials, almost impossible to perform. Smart theoretical efforts should come to reduce the space to be explored.

Additionally, the translation of complex system analysis to the clinical setting will also demand changes in the methodology of clinical trials themselves, bypassing the classical Phase I-II-III scheme and calling for multiple sequential pilot trials, each one with fewer patients but more measurements, combination of experimental treatments since the pilot trials and a permanent use of meta-analysis of several clinical experiments in data bases. This will also have an impact in the statistical procedures to be used, and probably also in the regulatory environment.

The master idea is to close the loop from computer simulations, to cellular and animal experiments, to the clinical trials, back and forth (Figure 2).

This is the focus of the Workshop "Immunotherapy for the New Century". Its discussions will revolve around cancer immunotherapy and autoimmune diseases, but it is probable that many of these concepts and propositions could also have impact in other fields of immunology such as chronic infections and parasitic diseases where the scarcity of vaccines and other immunotherapy breakthroughs is suggesting the exhaustion of the current paradigms of Immunology.

11. Jerne J. Towards a network theory of the immune system. Ann Inst Pasteur Immunol 1974;125c:435-41.

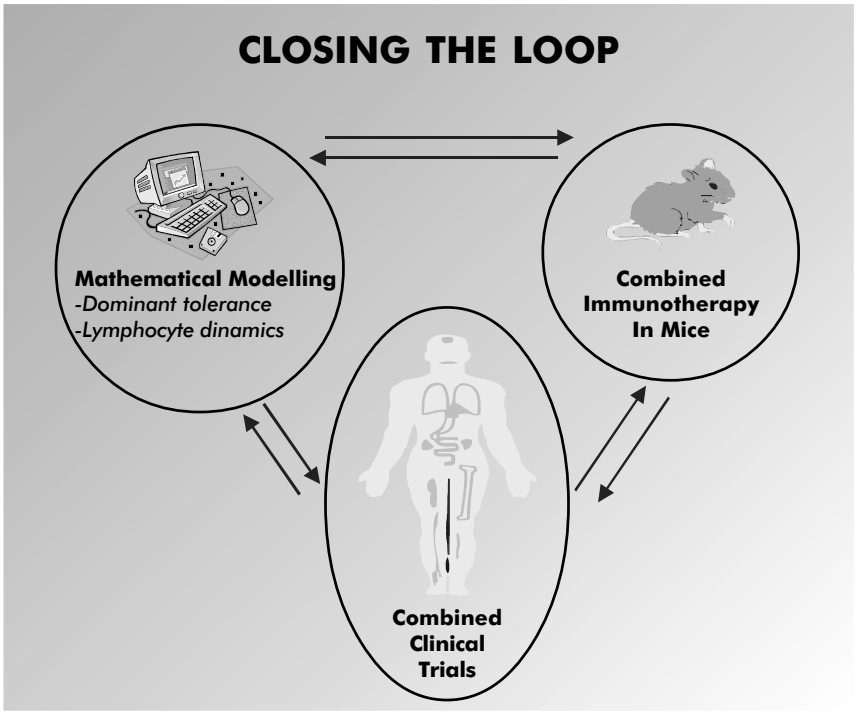

Figure 2. Mathematical modelling of the immune system, experimental immuntherapy and clinical immunotherapy are evolving separately. There is a source of advantage in creating two-way links among them.