



EDITORIAL

The specific immunotherapy of autoimmune diseases through the nanomedicine[☆]



El abordaje de la inmunoterapia específica de las enfermedades autoinmunes a través de la nanomedicina

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From time immemorial, mankind has tried to find cures for those diseases which cause the greatest morbidity and mortality.¹ One of the most significant examples is that of smallpox and its treatment, which was undoubtedly a great success worldwide. Variolization was initially performed for thousands of years in the East, and, after observations by Edward Jenner in 1796, was replaced by the systematic international application of a "vaccine" using the vaccinia virus. This eventually allowed the World Health Organization to declare in 1979 that smallpox had finally been eradicated.

The search for such "magic bullets" able to eradicate infectious agents while causing minimum side effects has long been one of the most sought for objectives in antimicrobial treatment. From the masterful work by Robert Koch and Louis Pasteur at the end of the 19th century leading to the first attenuated vaccines, and the research conducted by Paul Ehrlich and Alexander Fleming resulting in the discovery of chemotherapy for bacterial infections, to recent discovery of antiretroviral therapies and the generation of new recombinant vaccines, huge advances, decisive for the

health of mankind, have been made in the development of antimicrobial treatments.¹

This extraordinary explosion of knowledge in the field of antimicrobial treatments has also occurred in all aspects of medicine and the biological sciences. Immunology has been one of the disciplines where the most spectacular changes have occurred, partly because of the discovery of a method for producing monoclonal antibodies by Cesar Milstein and George Kohler in 1975.² Only 50 years ago, we only knew that there were two lymphocyte populations in the immune system: T cells, produced in the thymus and characterized by their capacity to form rosettes with sheep erythrocytes, and B cells, producing antibodies, whose ontogenetic origin in mammals was completely unknown. Today, thanks to an enormous international effort to classify monoclonal antibodies based on their antigen specificity in clusters of differentiation (CD), we know that there are an endless number of molecules involved in multiple physiological functions. The presence in cells of some of these molecules has allowed for the defining of different subpopulations of B and T cells, each of them with their own functional characteristics. For example, we know that there are two great populations of T cells, CD4+ and CD8+. We also know that there are different subpopulations of CD4+ T cells with different helper functions in the immune system. Thus, there are CD4+ Th2 cells able to induce the production of antibodies to B cells, CD4+ Th17

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cells whose function is neutrophil activation, CD4⁺ Th1 cells activating macrophages or cytotoxic CD8⁺ T cells, and regulatory CD4⁺ T cells (Tregs and Tr1 cells, among others) generated by various mechanisms with immune response suppression functions. On the other hand, CD8⁺ T cells with both cytotoxic and regulatory functions have been found. We also know that there are different B-cell populations, including follicular B2 cells, which when activated during an adaptive response are converted into antibody-producing plasma cells; B cells of the marginal zone of white pulp of the spleen, adjacent to the red pulp, mainly involved in innate immune response; and suppressor B cells (B10 or Bregs) with regulatory functions of immune response.

Today we also know that there are millions of different T and B cells which differ from each other in their unique antigen recognition receptors. In T cells, such receptors are called T cell receptors (TCRs) and serve to recognize antigens in the context of the molecules of the major histocompatibility complex (MHC). The MHC, also called human leukocyte antigen (HLA) in humans, consists of a set of polymorphic genes encoding membrane molecules of special importance in cell antigen presentation. Class I MHC molecules are constitutively expressed in all body cells, except for erythrocytes, and present antigens generated inside the cell to CD8⁺ T cells. On the other hand, class II MHC molecules are usually only expressed on the surface of professional antigen-presenting cells (APCs) and present antigens, mostly taken up from the extracellular space, to CD4⁺ T cells. The APC group consists of several cell populations of the immune system (dendritic cells, macrophages, and B cells) whose role is to activate T cells in the different immune response processes. To be able to activate T cells, APCs, in addition to presenting antigens in the context of MHC molecules, need to express other molecules, such as costimulatory molecules.

This extensive and complex world of molecules and cells is what finally determines our state of health and relates us with all that is foreign to it, differentiating this from what constitutes the self in a delicate balance. The loss of this balance may lead to a state of autoimmunity, i.e. a state in which the immune system responds to the self as if it were foreign to it and attacks it until it is destroyed. It is currently known that, during ontogenesis, T and B cells undergo comprehensive selection mechanisms that prevent the maturation of those with a high degree of self-reactivity. On the other hand, there are subsequent control mechanisms that prevent self-reactive lymphocytes which have escaped central selection controls from causing autoimmune responses once they are on the periphery. These cell control and selection mechanisms include: (i) "clonal deletion", i.e. the induction of death by apoptosis during the ontogenesis of lymphocytes with a high degree of self-reactivity; (ii) "anergy", occurring at both the central and peripheral level, by which self-reactive lymphocytes enter a functional state characterized by their inability to respond to the recognized self-antigen; and (iii) the transformation at central and peripheral level of potentially harmful self-reactive lymphocytes into "regulatory cells" that suppress an autoimmune response.

Once an autoimmune disease has been established, the prior balanced state is difficult to restore, and lifetime treatment is required in most cases. Either replacement

treatment for the deficiency caused by the autoimmune attack, such as exogenous insulin administration in type 1 diabetes, or immunosuppressant therapy to control this inappropriate response of the immune system may be used. Immunosuppressant therapies are indispensable for treating devastating autoimmune diseases such as multiple sclerosis, lupus erythematosus, or rheumatoid arthritis, but have a large number of untoward effects derived from their immunosuppressant activity. On the other hand, selective treatments intended to promote specific suppression have been unsuccessful to date.

In recent years, the research group directed by Dr. Pere Santamaria from the Julia McFarlane Diabetes Research Centre (JMDRC) at Calgary University, Canada, and the Institut d'Investigacions Biomèdiques August Pi i Sunyer (IDIBAPS) in Barcelona has designed a new approach to the treatment of autoimmune diseases based on "nanomedicine" using nanoparticles.^{3,4} Using this technology, these researchers have been able to generate the selective suppression of the immune response without affecting general immune system function, i.e. a treatment that restores the lost balance without affecting the state of immunocompetence of the subject. Nanoparticles are spheroidal structures less than 100 nm in diameter with physical-chemical properties able to modify physiological and/or pathophysiological cell responses.^{5,6} Their external structure, composition, size, and shape are essential for their cell and organ tropism. Materials used for the generation of nanoparticles range from carbon nanotubes to small metal balls coated with dextran or polyethyleneglycol to increase their solubility, all of them with specific molecules on their surface able to interact with ligands present on cell surfaces. There are other lipid-based nanoparticles which, after merging with the cytoplasmic membrane of cells, transfer to them the molecular charge they carry.⁷

The nanomedicine described by the group of Dr. P. Santamaria consists of iron oxide nanoparticles coated with polyethyleneglycol and molecular complexes of class I or class II MHC with autoantigen epitopes which are targets in autoimmune conditions. This therapeutic approach is based on the suppressive and/or regulatory effect on T cells of antigen recognition in the context of MHC molecules in the absence of costimulatory signals. Initially,³ they used iron oxide nanoparticles coated with molecular complexes of class I MHC with a peptide (epitope) of the islet-specific glucose-6-phosphatase catalytic subunit-related protein (IGRP), an autoantigen which is a target of autoimmune response of CD8⁺ T cells in type 1 diabetes in mice and humans, and induced disease prevention and cure in a NOD mouse model. Analysis of the therapeutic effect showed that this nanomedicine induced the systemic expansion of regulatory CD8⁺ T cells with a TCR with low affinity for the IGRP peptide which were able to induce APC suppression and/or death. The researchers concluded that APC destruction prevented subsequent activation of CD8⁺ and CD4⁺ T cells with TCR having a high affinity for epitopes derived from any other beta cell autoantigen taken up by APCs in situ. Unfortunately, the main limitation of this therapeutic approach was the difficulty in applying it in other autoimmune diseases. In addition, the existence of this population of regulatory CD8⁺ T cells in humans was not clear.

More recently,⁴ using nanoparticles coated with class II MHC molecules with peptides from autoantigens which are targets for autoimmune attacks in different mouse models of autoimmune diseases such as type 1 diabetes mellitus, multiple sclerosis, or rheumatoid arthritis, the same group clearly and conclusively showed that this therapeutic approach also induces the selective and specific suppression of the autoimmune response (a specific nanomedicine selectively designed for each disease) without affecting the general response of the immune system to other antigens. The results show that, in this case, unlike with the prior approach, a change is induced in the phenotype of self-reactive Th1, Th2, and/or Th17 lymphocytes to a regulatory phenotype of the Tr1 type with suppressor/regulatory capacity. The results also suggest that with the generation of Tr1, a cascade of cell events occurs, including the formation and expansion of regulatory B cells (Bregs), which synergistically with Tr1 induce the subsequent suppression of the pro-inflammatory activity of the APCs implicated in the promotion of the autoimmune response. It should be noted that this approach has a selective and specific therapeutic effect in all the models analyzed, which further emphasizes its importance as a potential future therapy for a large number of human autoimmune diseases. This extraordinary discovery may represent a U-turn in the treatment of autoimmune diseases, and a real breakthrough in medicine, although only time will tell whether it will be comparable with what antibiotic chemotherapy represented for the treatment of infectious diseases.

The next step will concern the use of this nanomedicine for the treatment of human autoimmune diseases. For this, however, the potential problems of large scale production will have to be solved, and undoubtedly will be overcome with advances in technology. Moreover, its effectiveness in an organism thousands of times greater than that of the mouse, more immunologically complex, and with a greater interspecies immunogenetic variability will have to be analyzed. In this regard, in order to assess its therapeutic potential in humans, the authors also analyzed the effect of this nanotherapy in humanized mouse models of type 1 diabetes, i.e. in immunodeficient mice reconstituted with mononuclear cells from patients with type 1 diabetes. Remarkable results were achieved, as all the treated animals

showed a clear expansion of CD4⁺ Tr1T cells and Bregs of diabetic patients with the regulatory/suppressor phenotype. The strength of these results clearly supports the therapeutic potential of this nanomedicine in humans and allows us to dream that, in the not so distant future, "magic bullets" for the treatment and cure of autoimmunity will indeed become a reality.

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Conflicts of interest

The author states that he has no conflicts of interest regarding this article.

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