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Transfer factor in the age of molecular biology: A review

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Abstract

Current data suggests that the transferring of immunologically specific information by transfer factor molecules requires interaction with a cell that has been genetically programmed to be antigen reactive but at the time of interaction is unprimed. Contact with transfer factor molecules would allow a naive recipient, on a first encounter with antigen, to make a secondary rather than a primary immunological response. Transfer factor molecules for each and every antigenic determinant are thus necessary. Transfer factors made from animals or humans are capable of transferring antigen specificity across a species barrier. Even primitive species have cells from which one can make transfer factors. The molecules are, therefore, well conserved and it is reasonable to suggest that they are important for normal immunological functioning. Proposed mechanisms of action must explain the fact that transfer factors obtained from the cells of high responder animals are capable of transferring delayed hypersensitivity to low responder animals while the reverse is not true. Transfer factor molecules are likely to interact with the variable regions of the alpha and/or beta chain of T cell receptors to change their avidity and affinity for antigen in a way that otherwise would only occur after an encounter with antigen.

Introduction

In 1905 the pioneering experiments of Clemens Von Pirquet allowed him to explore both the protection and damage that may follow a second encounter with antigen. He described the phenomena noted in terms of "useless allergy" and "useful allergy". The word allergy combined two Greek words "allos' and "ergos", altered energy. The useful allergy that he noted was what we today would refer to as "delayed-type hypersensitivity" while the dangerous and he thought "useless" immunological reactions he observed were clearly type IV or "immediate-type hypersensitivity" reactions.

Von Pirquet tried to explain everything in terms of antibodies for, in the early part of the century, the cell mediated immune system was yet to be understood. But pioneering experiments, begun in 1942 in New York, soon made it clear that the system was more complicated than the one proposed by Von Pirquet. It was noted that certain immunological reactions could be transferred from one animal to another by cells but not by serum and with this observation the second coming of cellular immunology was hastened and the concept of a division of labour between humoral and cellular immunity grew quickly. Antibodies mediated certain humoral immunity reactions, while the cells in the peripheral blood and spleen of animals that could passively transfer cell mediated immune reactions provided a unique repertoire of alternative immunologically protective reactions.

Further complexity developed when Lawrence in 1955 [1] demonstrated that delayed-type hypersensitivity to proteins derived from tuberculin, diphtheria and streptococci could be transferred from an immunised animal to an immunologically naive one by use of the lysates from sensitised peripheral blood cells. The era of "Transfer Factor" was born. The phenomenon described by Lawrence would have been interesting even if the transfer factors he discovered merely acted in a non-specific fashion to enhance some aspect of cell mediated immune performance, thus increasing the effectiveness of a recipients reaction to antigen. However, from the earliest experiments it was conceivable that transfer factors were taking immunological specific information from one animal and giving

it to another. Because of the difficulty in fitting such observed phenomenology into then current concepts the whole subject rapidly became controversial.

Lawrence and his co-workers soon realised that the molecular weight of the active portion of the transfer factors they were using was less than 10,000 Da. This made it even more difficult to conceive that immunologically specific information could be contained within such preparations. With the development in the late '60s and early '70s of evidence supporting the concept that any one T cell is genetically programmed to recognise one and only one antigenic determinant it became obvious that one would have to postulate that within preparations of transfer factors there must be a myriad of antigen- specific molecules.

It was of very considerable interest that the transfer of delayed-type hypersensitivity using cells required syngeneic donors and recipients if the transfer was to produce a long lasting response [2]. Transfer factors on the other hand were able to cross such genetic barriers. But a debate among immunologists concerning the antigen-specificity of transfer factors has at some times troubled and at other times entertained immunology since 1955. Amplification versus specificity arguments have been explored at length and both had their champions. As is so often true in matters of science and other affairs of men it appears that both concepts are valid. There is no doubt that in the sub 10,000 molecular weight dialysable leucocyte extracts that we are discussing there are non-specific immunologically active molecules that can stimulate, in a non-antigen-specific manner, various aspects of cell mediated immunity. Indeed, Gottlieb et al. [3] have purified two potent immunoregulatory molecules that are active at concentrations below 1 microgram per ml and have a molecular weight of less than 3,500 Da. These molecules are referred to as IMREG I and IMREG II. Both molecules have the same biological properties, but their dose response curves are distinctive: IMREG II in larger amounts is able to down regulate immune responses. These molecules can amplify but not transfer delayed-type hypersensitivity. Structural analysis of these molecules suggest that they are similar to the end terminal portion of MET and LEU Enkephalin. This pituitary hormone is known to have immunoregulatory effects. Interestingly and perhaps of significance for the discussion on antigen-specificity that follows, IMREG I enhances the expression of high density Interleukin 2 receptors (IL2R) [4].

Antigen-specificity

There is now incontrovertible evidence that some of the molecules within transfer factor preparations are able to transfer antigen-specific information. Indeed, current concepts based on recent data must accept that for every antigen there is a corresponding transfer factor molecule. The mechanisms by which these molecules transfer specificity has still not been elucidated, but with transfer factor molecules purified to homogeneity now available for study, molecular biology should allow us to rapidly unravel the mechanisms involved [5]. What follows is an analysis of the possible mechanisms of action that can be subjected to experimental testing.

Any examination of potential mechanisms must include an appreciation of our understanding of clonal selection. Under genetic pressure an enormous repertoire of antigen-specific cells is generated even before the birth of an animal. The human foetus at 16 weeks of age is capable of recognising more than 1,000,000 different antigenic determinants. Such recognition capacity has nothing to do with the presence of antigen. Receptors on T and B cells capable of having a "best fit" with only one antigenic determinant ensure that we can respond to any challenges, but at the same time limit a primary response to a somewhat inadequate reaction time, as there are relatively few cells that can respond to any one antigenic determinant on a first encounter. It is very likely that transfer factors are molecules that have evolved in an attempt to overcome some of the problems associated with the slowness of a primary immune response.

Transfer Factors would, in transferring immunologically specific information, be required to react with cells that have been genetically programmed to be antigen reactive. The only exception to this model would develop if transfer factors were found to be composed of sections of the T-cell receptor itself and, as such, were able to adhere to the membrane of recipient cells in such a way that they would in turn become passively antigen-specific. There is little evidence for this in the literature, although one experiment suggests that cells held in tissue culture which have lost their own antigen-specific receptors can become reactive to hepatitis B determinants after incubation with transfer factor specific for that virus [6].

It seems more plausible, however, that immunological specificity will involve the reaction of antigen-specific molecules derived from one host with the antigen-specific but unprimed cells of a recipient. Such

interactions would allow a naive recipient, when perceiving for the first time the presence of antigen in their ecosystem, to make a secondary rather than a primary immunological response. What is the evidence that transfer factor contains immunologically specific information that can be transferred from one animal to another?

Considerable difficulty has been encountered in proving the specificity of transfer factor molecules if the animals providing the transfer factors were immunised with ubiquitous antigens. When any recipients are tested with these same antigens they may have already experienced them in an immunologically suboptimal form. The IMREG effect might dominate and an illusion of antigen-specificity be provided. Transfer factors would, in that case, only be amplifying a suboptimal memory response. To address these problems, numerous experiments have been conducted in which transfer factors have been made from individuals who are highly sensitised to antigenic determinants that a potential recipient was extremely unlikely to have encountered. For example, in the United States, transfer factor was made from leucocytes obtained from individuals on the West Coast of that country who were strongly sensitised to coccidioidin. The preparation was then administered to volunteers on the East Coast of the United States where reactivity to coccidioidin among long time inhabitants of the area is virtually unknown. The successful transfer of delayed-type hypersensitivity to this antigen strongly suggested that antigen- specificity was involved [7].

Even more convincing experiments utilised the antigen keyhole limpet haemocyanin (KLH), an antigen to which no-one would normally be exposed. Blood was collected from volunteers for the production of transfer factors. These same volunteers were then immunised with KLH. Blood, collected after optimal delayed hypersensitivity responses had developed in the donors, was used to make a further batch of transfer factors from the same individuals. In a double blind study, pre-KLH and post-KLH transfer factors were administered to volunteers. Twenty five of the 26 recipients of transfer factors made after KLH immunisation where able to respond to KLH [8]. In reviewing literature on this subject, one can see that experiments attempting to show antigen-specificity have demonstrated that antigen-specific transfer factors have a 78% capacity to transfer delayed-type hypersensitivity, while control preparations cause an apparently antigen-specific response in only 7.9% of recipients [9].

From this and other evidence, the concept has developed that there will be transfer factor molecules for each and every antigen; in this sense they would be analogous to antibodies. It should be noted, however, that there is no evidence that transfer factors interact with the humoral immune system in any way.

Transfer factors can be made from animals or humans and are capable of being administered across a species barrier without any loss of potency. Even primitive species have cells from which one can make transfer factors that will be effective in higher species. Clearly these low molecular weight molecules have been very well conserved for millions of years, pointing to the importance they play in the immune response. Indeed, in thinking about transfer factors, it is critical to analyse the role they would normally play in the individuals whose immune system produces them, for only when we understand their in vivo effects for their producer will we be able to comprehend the way in which they may help a recipient.

Do transfer factors contain antigen?

If transfer factors contained even small antigenic determinants, then its specificity could be explained. Their low molecular weight pleads against this, but with the realisation that most antigenic determinants are recognised as peptides, containing between 15 and 17 amino acids, this possibility needed to be examined carefully. Perhaps, the single piece of evidence that argues most strongly against the concept of antigen being present in transfer factors, is the demonstration that adequate delayed hypersensitivity responses can frequently be generated within 12 hours of an injection of transfer factors. Immunisation with antigen, in even super optimal amounts, would never be able to induce such a rapid immunological response.

More recent experiments have almost eliminated the possibility that transfer factors contain antigenic determinants. The antigen-specific properties of transfer factors can be removed by incubating preparations with a specific antigenic determinant before administering it to a recipient. In both animal and human models, utilising both in vivo and in vitro systems, this phenomenon has been constant. If you pass transfer factors over antigen coated beads, using a preparation made from an animal immunised to more than one antigenic determinant simultaneously, the transfer factors that are passed over the beads will only be able to transfer delayed-type hypersensitivity to the antigen

not coated onto the adsorbent. If you elute the transfer factors from the antigen coated beads, the inability to transfer delayed hypersensitivity to a second or third antigen will be restored [10].

The response to many antigens in different strains of laboratory animals allows us to examine the concept of high responder and low responder strains. Some animals, given a specific dose of antigen, will make an excellent humoral and cell mediated response to that challenge. Other animals, exposed to exactly the same antigen make a poor response. Transfer factors, made from the cells of high responder animals, are capable of transferring delayed-type hypersensitivity to low responder animals, but the reverse is not true [11]. This phenomenology provides very strong evidence against the presence of antigenic determinants in the low responder strain transfer factors. One would obviously expect high responder strains to have no trouble responding to antigenic determinants, if they were present.

Chemical properties

Recent data make it likely that the antigen-specific molecules found in transfer factors have a molecular weight of less than 5,000 Da. Some investigators have suggested that transfer factors are composed of a polypeptide and may consist of more than one chain held together by a di-sulphide bridge. Others have produced evidence suggesting that attached to the polypeptide chains are nucleic acids or phosphodiesters. The transfer factor molecules recently purified to homogeneity however appear to be composed entirely of amino acids [5]. One of the more reliable observations made by investigators of the antigen-specific properties of transfer factor molecules has been the demonstration that shortly after an injection of a preparation, cells in the recipient can respond to antigen in vitro by the production of macrophage inhibition factor (MIF). There is a considerable amount of evidence to suggest that MIF is in fact gamma-interferon; indeed, studies have suggested that interferon-like activity can be found in the serum of people shortly after an injection of transfer factors [12].

Controversy still surrounds the question as to whether transfer factors, when transferring delayed-type hypersensitivity capacity, allow antigen- specific CD4 cells of a recipient to proliferate. It appears that CD4 T lymphocytes provide the major antigen-specific response required to produce delayed-type hypersensitivity. There is some evidence in the literature suggesting that cytotoxic CD8 T lymphocytes can expand after encountering specific transfer factors when given to patients with osteogenic sarcoma [13]. It is possible, however, that the lack of CD4 expansion could explain why it is that memory associated with transfer factors is somewhat limited. Seldom do antigen-specific reactions persist for longer than six months. On the other hand, it is difficult to explain, if there is no expansion of CD4 T lymphocytes, how it is that transfer factors produced by recipients of transfer factors can readily transfer specificity to a third party. Indeed, serial transfer experiments have been successfully completed on a number of occasions. Recent studies have suggested that, perhaps, there are molecules within dialysable leucocyte extracts that are able to interfere with normal immunoregulatory suppressor cell loops which normally minimise a primary response. It has been suggested, for example, that transfer factor molecules may activate contra-suppressor cells, which would in turn suppress immunoregulatory CD8 T lymphocytes, thus allowing an enhanced response to a first encounter with antigen [14]. No evidence is available to support this concept.

If the molecules under discussion were in fact part of a shed T cell receptor, then such molecules could in some way be able to interact with antigen or other T cell receptors of a recipients antigen-specific T cells, thus initiating an immunological reaction. It is of interest that the antigen-specific nature of transfer factors can be removed by incubation of these molecular mixtures with antisera against the VH region of immunoglobulin heavy chains. As these same molecules bind to segments of the T cell receptor, such observations suggest that transfer factor molecules may contain part of the antigen-specific T cell receptor [15].

Do antigen-specific transfer factor molecules work through an anti- idiotypic mechanism?

After an encounter with antigens, T cells may release an anti-idiotypic molecule capable of activating antigen-specific T cells that have not yet seen antigen. In so doing, they may alter the membrane characteristics of the T cell receptor, so that it is expressed on the surface of the cell in a secondary rather than a primary mode. An increased avidity and affinity for antigenic determinants could result in recipients having the equivalent of primed cells circulating efficiently throughout their body, before they have actually

encountered the relevant antigenic determinant. Such an amplification would make immunological sense. The concepts related to such phenomenology are supported by the observations reported above, wherein IMREG is capable of increasing the expression of IL2R on the surface of a recipient's cells. It would be interesting to look at the DR status of those cells, capable of responding to antigen in any way after the passage of antigen-specific information by transfer factor molecules.

While much is still to be learnt about the immunological mechanisms provoked by the transfer of these low molecular weight dialysable molecules, in an age when we know of so many messages being passed from one cell in the immune system to another by low molecular weight interleukins and the diminutive nature of antigenic determinants, it is clear we should be less surprised that small molecular weight substances can be antigen-specific. Given the techniques now available to molecular immunologists, it may well be that the remaining major immunological mysteries associated with the very powerful properties of transfer factor molecules will soon be understood. Certainly the study of the phenomenology is likely to teach us much about the immune system in general and provide us with new ways of approaching, in a uniquely safe manner, therapeutic manipulations of the immune system of patients with immunological diseases.

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