

QUESTIONS ON SELF REGOGNITION AND AUTOIMMUNITY IN CHRONIC CHAGAS DISEASE: AN IMMUNOLOGIC APPROACH.

SY-4

George A. Dos Reis. Departamento de Imunologia. Instituto de Microbiologia da UFRJ, Cidade Universitária, Ilha do Fundão, Rio de Janeiro, 21.944, Brasil.

Introduction

Researchers dealing with parasitology and immunology of Chagas disease are well acquainted with the problems imposed by chronic, often progressive, tissue lesions which appear in heart and digestive tract of individuals infected with Trypanosoma cruzi. Given the high incidence and severity of symptoms in the afflicted population, this life-threatening condition is a serious Public Health affair for many Latin-American countries. Although reminding all this suffering, I will turn to the biological problem. We all expect someday we can go back to the real people with something good we learned, to offer. It is not my purpose here to review the data, not even the most widely accepted theories to explain immunologic processes leading to chronic phase of Chagas' disease. Two excellent and authoritative reviews on the subject have been presented last year in this meeting by Drs. Z. Brener (1) and G. Schmunis (2), and should be carefully studied by anyone willing to get a deep insight into this fascinating and yet, sometimes discouragingly unaccessible, research subject. My purpose is to formulate some few questions, in the light of basic knowledge in lymphocyte immunobiology. It is my obvious hope that any effort in trying to answer my questions, would make the overall subject simpler. However, I am aware and prepared for the more realistic possibility that such answers may add to the complexity, instead.

Autoimmunity hypotheses, but no formal proof

This is not a criticism. Actually, the situation of what is known about the cardiac form of Chagas' disease is not different from many other autoimmune diseases. Ignorance and confusion still prevails, behind a scene of flash lights brought about by episodic, discoveries, which often turn out to be elusive Pandora's boxes in our enthusiastic, usually medical-minded, crusade against disease. If nothing, we learned a lot about T. cruzi-host cell and T. cruzi immune system relationships, which certainly will add to a general improvement of our knowledge on the biology of

target cell. Accordingly, it has been shown that, if aggregation is not extensive, lymphokine production is a polarized process, occurring exactly at the site of CD3:TcR aggregation. Although our knowledge of the immune response has expanded with studies on lymphokines, this progress is restricted by our lack of information about the cell biology of lymphoid cells. Exact and informative as they are, "in vitro" experiments do not help us to understand the real "in vivo" situation. For example, one is left with little information on how extensive is CD3: TcR aggregation in vivo. We don't know what are the "in vivo" amounts of accessory cell-associated antigen, and we don't know whether IL2 production is a relevant "in vivo" phenomenon or not. Studies with T cell tolerance induction in the adult, revealed that absence of an appropriate "second signal" coupled with T cell receptor occupancy leads to T cell tolerance, rather than priming (6). Tolerance is not absence of response, but rather, an alternative and truncated mode of CD4⁺T cell activation, with long-lasting inactivation of the IL2 gene, but with activation of genes for other lymphokines. In addition to antigen and tolerogen, T cell biology may be modified in vitro by anti-TcR antibodies. However, evidence that autologous anti-Id Abs play a role in "in vivo" regulation of immune responses, at their physiological low dosages and affinity range, is scarce, except perhaps in the case of transfer of idiotypic connections by milk.

Could anti-Ids be capable of inducing "in vivo" CD3: TcR aggregation? Perhaps they synergize with other physiological modes of cell interactions, such as anti-MHC Class II autoreactive T cells. Although these are still open questions, the important point to be emphasized, is that an old fashioned way to think of a lymphocyte response as a consequence of an all-or-none response to a single ligand is wrong. It must be reevaluated by quantitative thinking about integrative properties of T lymphocyte membranes given by cognitive and alternative (cell adhesion molecules) signalling pathways- if we want to approach reality closer than before. The living mouse or human being may have immune systems, whose logic is quite different from what we are used to symbolize with simple and single "Ys", triangles, and receptors, or also with "a priori" conceptions that any antibody, at any concentration, will have an effect on the cell with which it is interacting.

What would we like to know about autoimmunity in Chagas' disease?

Of course, we would like to know if it exists, at least, as an active cause of disease. For an autoimmune process to take place,

there must be appropriate anti-self heart T cell clones. I will not doubt that anti-self heart antibodies exist. At least in this area of Chagas' disease research, sound evidences for antibodies directed to laminin, nidogen, and a sarcoplasmic ion transport enzyme exist (reviewed in 2). They might have however been produced as a result of polyclonal B cell activation. Auto-antibodies are common features of a normal and healthy immune system, which seems to cope with autoreactivity quite well, without leading to noxious injury. There is no evidence that these antibodies, although augmented, play any role in initiating cardiac injury. Because of these and other compelling evidences from other models of autoimmune disease, we should look into T cells. Our group investigated anti-self heart T cell clones in mice immunized and infected with *T-cruzi*, without success (7). Anti-heart T cells we detected were directed to allogeneic MHC molecules present in the heart antigen material, and have been primed " in vivo " by infection with *T-cruzi*, as noted by their accelerated kinetics on the proliferative responses. This finding is not surprising, giving massive T and B cell activation occurring "in vivo" after *T-cruzi* infection (8) . This failure to detect such autoimmune heart- reactive T cells contrasts with our previous studies with streptococcus-primed mice (9) . Thus, we concluded that anti-self heart T cells cannot be directly expanded by *T-cruzi* . More sensitive techniques to detect autoreactive cells were not employed. Limiting dilution analysis, for example, could detect cells under suppression because one may dilute suppressor cell activity before diluting autoimmune T cell clones. Also, elimination of $Lyt2^+$ cells may help to detect such cells, if they exist. In addition, assessment of T cell activation with RNA or DNA probes, or measure of other lymphokines distinct from IL2, could be more sensitive ways of detection. Of course, these experiments are waited, to see what the answer is. On the other hand, we have identified anti-self heart T cell responses in a proportion of the chronically infected animals. The animals were inbred, so most probable explanations for this finding were self recognition as a consequence of an erratic process of tissue injury, or as a consequence of unequal idiotypic regulation among different animals. Certain patterns of idiotypic regulation are usually a characteristic of some, but not all animals in a given strain. Besides the responses being feble, this individual variation adds more difficulties to the issue. Positive animals should be tested without sacrifice, in order to follow them clinically, in comparison with negative ones. The finding of anti-myosin T cells, not crossreacting with *T-cruzi*, but present in infected mice (see Rizzo,

Cunha Neto and Teixeira, this symposium) is interesting, and also suggests "autoimmunity" as a consequence of primary heart cell injury. The question is whether these T cells against intracellular components of heart tissue could have a pathogenetic role in maintaining the disease or, in other words, whether is this self recognition or true autoimmunity. Of considerable importance to test pathogenetic theories related to certain anti-self T cells, would be the development of rapid, simple and quantitative techniques to detect heart cell injury in an experimental "in vivo" model. One intracellular enzyme of cardiac muscle was able to give information on massive lesions in murine Streptococcal carditis (9), but individual variation in controls is high, making it difficult to assess discrete injury at early steps of the chagasic process in mice. Other enzyme or protein markers of the heart tissue should be evaluated, in order to provide simple and accepted standards of what an initial lesion is. Histopathology alone, discrete as it is, and "post-mortem" in the mouse, is not the solution. One report last year (10) suggested an interesting way to follow cardiac function in experimental mice: implant of syngeneic heart in the ear's tissue of the recipient.

Another central question is related to the nature of the original antigenic determinant initiating attack. By definition, it must be expressed at the surface of a heart muscle cell, it has to be a fragment of a heart structure, and should be complexed to MHC Class II (probably) or Class I molecule, to be recognized by T cells. For example, Thyrocytes and pancreatic islet cells express intrinsic MHC class II molecules in disease models, as a result of combined action of γ -IFN and TNF or lymphotoxin, which are products of activated T cells and macrophages (3). In human disease too, aberrant expression of MHC Class II is evident (3). This is thought to be necessary for the target autoantigen to be presented to the appropriate inducer T cell and the process to be perpetuated. It might be argued that the cardiac determinant mimicks an anti-idiotypic. It is not clear, but rather doubtful, whether anti-Ids could activate T cells in the absence of MHC recognition. If nothing, such contacts in the absence of a second signal, should tolerize the T cell. There is no description yet, that heart muscle cells would express Class II MHC following cytokine treatment. Also, there is no "insitu" study to investigate whether heart cells are expressing MHC Class II at the site of lesion in Chagas' disease. These are important questions to be investigated. Finally, for T cell activation to occur, cell adhesion molecules must also be expressed by the target cells. We, thus, wait investigations on expression of ICAM-1 (ligand for LFA-1), Thy 1,

and LFA-3 in heart muscle cells, specially after T-cruzi infection, or after cytokine treatment. If these molecules, or any correlate of them, are not expressed, I would doubt that lymphocyte-mediate injury could really take place in chronic disease, for there would be no way to activate T cell responses against those targets. Another aspect which has not been discussed is the possibility of CD8⁺ T cells being the effectors of the initial attack. Although these cells usually proliferate in current assays, one cannot discard the possibility that T cell effectors in Chagas' disease are non-IL2 (or other lymphokine) producers, and that they rely on joint recognition of another epitope, perhaps of T-cruzi origin, by CD4⁺, IL2- producing, stand by helpers, in order to differentiate and or to develop effector function.

Several interesting and related questions about the chronic phase of disease are linked to studies on the T cell repertoire. But lack of a reliable and quantitative assay of heart cell lesion in model systems has prevented studies on genetic variability of different strains upon chronic injury following T-cruzi infection. Histopathologic examination gave no indication on genetic control of these manifestations, although it is hard to compare different aspects of cardiac damage in terms of subjective examination of tissue sections. Model systems where one can measure the weight of an organ or the thickness of a lesion site have proved more amenable to genetic investigations. One question is related to whether changes in the immune system during acute infection determine the later chronic outcome of a degenerative process in heart. There is a report that severity of the chronic process is related to the severity of the acute phase (1). If quantitative studies could be performed on the extent of cardiac damage in chronic stage, it would be interesting to correlate this stage to several degrees of extension of the acute phase, by experimentally reducing acute manifestations with chemotherapy. One important group in the area champions the idea that the impressive activation of T and B lymphocytes at acute stages, may be the cause of radical regulatory changes in the immune network which, consequently, lead to autoimmunity in chronic stages (9). In fact, other areas of autoimmune disease research, lend support to the idea. Irradiation plus thymectomy, or repopulation of athymic nude mice with lymphoid cells depleted of certain T cell subsets, lead to concomitant appearance of severe forms of autoimmune disease in thyroid, gastric mucosa and oviduct in mice, closely resembling human diseases (4). Also, it has been repeatedly reported that autoimmunity appears after syngeneic transplants following

cyclosporin A treatment (11). This latter condition can also be viewed as a radical modification of the host T cell repertoire. These studies are strong support for the natural occurrence of potentially autoimmune T cell clones in the normal host, and for the notion that autoimmunity is normally prevented by the individual solution of each host to its own mature T and B cell network connectivity. Any radical modification of this connectivity caused by acute infection with T-cruzi may result in autoimmunity before the host is able to restore an overall " healthy " or balanced conformation of its idiotypic web of interacting cells. Why should autoimmunity be centered on heart tissue, and not on stomach or thyroid, in these conditions, is not clear yet. A very interesting approach to study of lymphocyte repertoires in T-cruzi infection is the recent demonstration of a role for Ly 1 B cell population (which is enriched in peritoneum, and participates in natural antibody production not related to extrinsic antigenic stimulation) , and enhanced activity of CD4⁻ CD8⁻ CD3⁺ T cell function in spleens of mice infected with T-cruzi (see Minoprio and Coutinho, this Symposium). This unusual T cell subset is also expanded in well known murine T cell- dependent autoimmune disorders. The finding that acute T-cruzi infection can be detected in thymus of mice, with massive depletion of thymocytes (12) suggest that additional and rather important changes in the host lymphocyte repertoire take place during infection. It is unknown whether this could favor autoimmunity. If quantitative assessment of the chronic stage becomes available, interesting experiments combining T-cruzi infection with repertoire modification by irradiation, depletion of subsets, or cyclosporin can be made to evidentiate autoimmune process.

Conclusion

I have emphasized the need for a better model system, and for quantitative monitoring of heart damage in mice during chronic Chagas' disease. I think all efforts should be done to improve the mouse as a model system, because most of what immunologists can do with lymphocytes, now and in the near future, can only be done in the mouse. In spite of the skepticism, I think that many of the ideas I have presented (and certainly many others) are also the ideas of several researchers in the area. It seems that a general improvement of our knowledge about chronic Chagas' disease is possible, by all means. The only necessary breakthrough is, in my opinion, a methodological one . Of course, autoimmunity is a popular idea in Chagas' disease. Some people cannot conceive alternative ways to view this process. If immunologists were to be out of business in the subject, what kind

parasites and mammalian system. Does T-cruzi produce substances capable of inactivating or modifying the biology of certain cells, such as muscle cells or neurones? Does early neurone damage lead to progressive cardiac disfunction? Could antibody against a hormone or neurotransmitter receptor be the first causative agent in the pathogenesis of the process? Is there a role for cardiac damage and subsequent immune responses in perpetuating an auto-aggressive process? Is there a true autoimmune disease, in the sense that it can be propagated to a normal recipient by sterile transfer of antibodies or T cells? Is there a causal link between early alterations in the immune system during acute phase, and the subsequent outcome of the chronic disease? Is the chronic process allergic, autoimmune or still dependent on existent nests of parasites? As you could note, all of these questions and many others you may remind have been experimentally approached (1,2), answered, and subsequently disputed, so that we are now left with little more than nothing. Lack of an appropriate "in vivo" experimental model (both for the disease itself, but also for immunologic maneuvers) is a real obstacle. However, in some other cases of autoimmune disease, with more adequate models, some progress has been clearly made. (3,4). Those more or less recent advances suggest new areas where Chagas' disease research should be directed. Other concepts in basic immunobiology, not necessarily related to autoimmunity, may equally contribute to improve our knowledge.

T cell activation, lymphokines and target cells

To be activated, T cells must have their CD3: TcR complexes aggregated in the plane of the membrane (5). This process leads to stimulation of phosphatidylinositol turnover, increases in free Ca^{2+} in cytoplasm and protein kinase C translocation to the membrane; These early events are linked to onset of lymphokine synthesis and expression of cell surface receptors for growth and differentiation factors (5). Cell adhesion molecules play an essential role in T cell activation, as blockade of these structures with monoclonal antibodies, or defective expression leads to little or no antigen-directed T cell activation. Cell adhesion molecules include CD4, CD8, CD2 and LFA-1 in man, and they seem to provide a cooperative set of interactions leading to stable conjugate formation between the T cell and the target cell, so that a synaptic cleft is formed, ensuing very high local concentrations of lymphokines, which could then interact with the

of specialist would pathologists require? Enthusiastics of experimental medicine and pathology should not refuse to think about general metabolism and physiology of heart cells in normal and infected hosts, for the inflammatory process could still be of non-autoimmune or non-allergic origin. It is well recognized that certain dogmas happen to impede, rather than to foster the advancement of science. It seems that all evidence presented so far for autoimmune involvement in the chronic stage, has been disputed. Thus, the evidence hasn't been overwhelming, as it should, and as it is in other autoimmune disease models. That raises the possibility that the whole idea is wrong, and is impeding progress in Chagas'disease research. However, the data were actually collected by independent groups. Their poor quality may be an unfortunate consequence of a bad model system, and of bad ways to quantitate and compare the results. Perhaps, it is not occasional, that the most fundamental and new information in experimental Chagas'disease, is coming from a physiological approach to changes in the whole lymphocyte repertoire during infection. To correlate this new concept with the chronic stage, however, will require a precise and objective quantitation of late heart tissue injury. I think we face the challenge of optimizing the available models, and of applying recent concepts in basic immunology before preparing the final attack to such an embarrassing question of whether is there an autoimmune origin in chronic Chagas'disease.

References

- 1 - Brener, Z., Mem.Inst. Oswaldo Cruz, suppl.vol 82, 205,1987.
- 2 - Schmunis, G.A., Mem.Inst.Oswaldo Cruz , suppl.vol 82,287,1987.
- 3 - Feldmann, M., Mem.Inst. Oswaldo Cruz, suppl,vol.82,274,1987.
- 4 - Taguchi, O. and Nishizuka, Y., J.Exp.Med. 165, 146,1987.
- 5 - Alcover, A., Ramarli, D., Richardson, N.E., Chang, H.C. and Reinherz, E.L., Immunol.Rev. 95, 5, 1987.
- 6 - Jenkins, M.K., Pardoll, D.M., Mizuguchi, J., Quill, H. and Schwartz, R.H.,Immunol.Rev. 95, 113, 1987.
- 7 - Gattass, C.R., Lima, M.T., Nóbrega, A.F., Barcinski, M.A., and Dos Reis, G.A.,Infec.Immun. 56, 1402, 1988.
- 8 - Minoprio, P.M., Eisen, H., Forni, L., D'Imperio Lima, M.R., Joskowich, M. and Coutinho A., Scand. J. Immunol. 24,661,1986.
- 9 - Dos Reis, G.A., Gaspar, M.I. and Barcinski, M.A., J.Immunol. 128, 1514, 1982.
- 10 - Laus, J.L., Rossi, M.M.O. and Ribeiro dos Santos, R., Mem. Inst.Oswaldo Cruz. suppl.vol 82, 168, 1987.
- 11 - Glazier, A., Tutschka, P.J., Farmer, E.R. and Santos, G.W., J.Exp.Med. 158, 1, 1983.
- 12 - Leite de Moraes, M.C. and Savino, W., Mem. Instituto Oswaldo Cruz, suppl. vol. 82, 114, 1987.

Acknowledgements: Author's work cited was supported by W.H.O./
TDR, FINEP and CNPq.