Do stress proteins have a regulatory role in autoimmune Rheumatoid Arthritis?

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It has been suggested that molecular mimicry between self-(mammalian)-components, such as self-stress, heat-shock proteins (Hsp60 & bacterial Hsp65), Mycobacterium (M.) tuberculosis or M. bovis, might be implicated in the human rheumatoid arthritis (RA) – an autoimmune inflammatory joint disease, which is characterized by infiltration of lymphocytes— predominantly of the T lineage in the synovial fluid (SF) & of the T & B lineages in the synovial membrane.

The observation that higher frequencies of T-cells, from RA individuals relative to the T-cells from healthy individuals, can recognize the self-stress proteins, & the observation that infections with Mycobacterium (in which a link has been found between inducible Hsps & virulence, & where the induced Hsp can be regarded as a virulence function in immune surveillance & defense against the bacterial infection) provide a basis for suggesting that the bacterial Hsp65 has a potential involvement in the induction of the human autoimmune RA.

On entering the mammalian host, pathogenic bacteria might induce a heat-shock response & activate the oxidative stress of the innate immune system phagocytes -functioning to clear the pathogens. As a balance must ensue to allow the induced Hsps to be superimposed on the regulatory mechanisms controlling the bacterial adaptability to the host stress, as well as the host survival, the Hsps expressed in innate immune system phagocytes -functioning to clear the pathogens, can be regarded as virulence & where the induced Hsp65 epitopes share some 50% sequence similarity, overstimulation by infection might also alter the balance of the immune response to the self-stress proteins & lead to destructive surveillance, to autoimmunity. In other words, the homology between the conserved epitopes of the bacterial Hsp65 & the self-Hsp60—that implicate Mycobacterium Hsp65 in manifestations involving potential antigenic mimicry (11)—fails to extend to immunological tolerance.

Different Hsps distinguishable on the basis of the molecular mass, are grouped into families denominated: Hsp60(65); Hsp70(75); Hsp90, each of which subserves, apparently, different functions. The Hsp60(65) & Hsp70(75) multigene family members function as molecular chaperons assisting protein folding & assembly—by reducing the tendency of aggregation during various cellular processes & as such are required for survival. That the conserved Hsp60(65) & Hsp70(75) are expressed ubiquitously in prokaryotes & eukaryotes (from bacteria & yeast to mouse & man), both constitutively (in the absence of any kind of stress) & inducible (1) can make them biologically meaningful mimicry elements.

Why has Hsp65 been implicated in the pathogenesis of RA? RA is a joint disease that is autoimmune in nature, caused by the host immune defense turning against a self-Ag (autoAg), present presumably in the synovial joint & that might be collagen? The consequences of the disease are the destruction of the joint (that seem to reflect host immune response to self-Ag), because autoantibodies (autoAbs) against collagen & autoAbs against other self-components are a feature of the RA [2].

Some reactivity against self-components is a common feature of the immune system since, under normal physiological conditions, the family of pathogen/Ag-recognition & binding receptors, which comprise the immunoglobulin receptors anchored onto the membrane of the B lineage cells (the BCR) & the T-cell receptors (TCR) expressed on the T-cells, are generated by rearrangement & random combinatorial associations of the variable gene segments & as such, must comprise along with the specificities recognizing foreign pathogenic Ags, also specificities that are capable of recognizing self— to mount a destructive immune response against self. But generally, mechanisms operate within each individual to ensure that the emergent repertoire of the B- & particularly of the T-cells, is tolerant to self. But in the susceptible RA individuals, elevated autoAbs against self-cytoskeleton components, such as intracellular nucleic acids, ssDNA & ddsDNA & histones, & Abs reactive with microbial stress proteins, Hsp65, have been delineated, suggesting that the microbial world might be implicated in the pathogenesis of RA. Most autoimmune diseases are mediated by sustained B- & T-cells adaptive immune responses: secreted autoAbs binding to the BCR & TCR receptors, either stimulating the cells further, or blocking further stimulation by the corresponding or by cross-reactive ligands.

To dissect the potential for mimicry between the bacterial components & self-components that might provide that link between anti-microbial immunity & autoimmunity, herein, I dissected the T-cells derived from RA patients, for reactivity motifs in 3-4 day culture with whole M. bovis extract, or with attenuated M. bovis BCG, or with M. bovis purified Hsp65, or with mammalian purified Hsp60, or with soluble purified protein derivatives (PPD) --that might support mimicry.

Figure 1: depicts the in vitro proliferative/activation responses to Hsp65 & PPD, of the mononuclear cells (MNC) separated from paired peripheral blood (PB) & SF samples derived from RA patients (A, C, D), relative to the responses of the MNC separated from PB derived from patients suffering from autoimmune diseases other than RA, such as Hashimoto’s thyroiditis (HT) (B), 86, & relative to the responses of the MNC separated from PB controls with no clinical symptoms of autoimmunity (B1). The reciprocal frequency x10 of the responses to Hsp65, of the MNC derived from the HT patients was between 1,500 & 3,500, & that of the MNC derived from the controls was between 500 & 1,900. B, C, D, depict the phenotypic motifs of the MNC T-cells derived from RA patients, that are reactive with the Mycobacterium Hsp65. (B3) depicts MNC T-cells derived from an HT patient who also had RA; 9B depicts MNC T-cells derived from an HT patient who did not have RA; 91 & B0 depict control MNC T-cell.

PB MNC were separated from erythrocytes & debris by Ficoll-Hypaque density gradient sedimentation of heparinized blood collected (for diagnostic reasons) from subjects that either met the American Rheumatism Association 1987 revised criteria for RA, or for non-RA, PB SF was aspirated for diagnostic & therapeutic reason in heparin & the MNC obtained from these separations by centrifugation (at 3,000 rpm) were deprived of phagocytes by carbonyl iron ingestion & removal in magnetic field & purified by ficoll-Hypaque density gradient centrifugation. Synovial tissues (ST) were placed in Ca2+ & Mg2+-free Hank’s balanced salt solution, minced & enzymatically digested for 2h at 37°C with collagenase 1mg/ml & DNases 0.1 mg/ml, & separated on discontinuous Percoll gradients (10%, 20%, 50%, 60%) to obtain the T-cell enriched fraction that sediments between the 50% & 60% Percoll layers. For proliferative assays cells (105/well) were cultured for 72 h, in flat-bottomed 96-well microtiter plates, in the presence of Hsp65 (1ug) or PPD, in modified Dulbecco’s medium supplemented with pooled human sera (10%), penicillin & streptomycin & for 16-18 h, in the presence of added titrated [3H]thyminde.

Results of the incorporation of [3H]Thyme (measured in a scintillation counter), & results of the phenotype motifs of the T-cells expanded from the various RA & controls in culture, as assessed by flow cytometry in terms of the cluster differentiation (CD) Ags (CD3, CD8): cluster activation Ags (CD57; CD45RO; HLA-DR): T-cell clonality (TCR Vβ7.1), suggest potential mimicry, the cross-reactivity between the bacterial Hsp65 & self-Hsp65 potentially controlling the balance of the regressive T repertoire.