HYPOTHESIS

Heat shock proteins: the missing link between hormonal and reproductive factors and rheumatoid arthritis?

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There is strong evidence for a link between endocrine and reproductive events and rheumatoid arthritis, not only in relation to disease activity and progression but also, and more intriguingly, with its incidence. Although some controversy still surrounds the significance of the epidemiological data, various studies suggest that a several fold increase or decrease in the risk of rheumatoid arthritis is dependent on the influences of such factors.

This clearly indicates the need for a strong research effort in this area, but it has been difficult to establish an acceptable biological explanation to guide investigation. It is the purpose of this article to propose a possible mechanism of interaction between the disease and the hormonal factors thought to affect it.

Rheumatoid arthritis, parity, and oral contraceptives

Several clinical and epidemiological studies strongly suggest an important role for sex hormones in the incidence and evolution of rheumatoid arthritis. The disease is about three times more prevalent in women than in men,^{1 2} but the difference tends to be greatest during the last reproductive years and reduces with advancing age thereafter.³

Reproductive aspects have also been related to the risk of rheumatoid arthritis: nulliparity has been found to be an important risk factor for the disease in a number of studies, being associated with a relative risk of 1.5 to 1.8 in comparison with women with a history of one or more pregnancies.⁴⁻⁷ According to most of the studies increasing numbers of pregnancies are not associated with further protection.

Unfortunately, these studies do not clarify the direction of this relation: the higher prevalence of rheumatoid arthritis in the nulliparous might be simulated by a reduced fertility in patients with this disease as shown in other autoimmune conditions.^{8 9} A number of studies on the possible relation between rheumatoid arthritis and poor reproductive outcome have been reported. Most of these studies¹⁰⁻¹² found no differences in miscarriage and stillbirth rates between patients with the disease and controls. Kaplan, however, reported a 50% increase in the rate of miscarriage among women with rheumatoid arthritis compared with controls with osteoarthritis, though there was no difference in overall fertility.¹³

Recently, much interest has been generated by the possible protective effect of oral contraceptives against the subsequent development of rheumatoid arthritis. The issue was raised by the unexpected finding, in a large study on the use of oral contraceptives,¹⁴ that the relative risk for rheumatoid arthritis was 0.49 (95% confidence interval (CI) 0.29 to 0.83) for current users and 0.68 (95% CI 0.45 to 1.03) for those who had ever used hormonal contraceptives in the past, in comparison with patients who had never been exposed to these hormones.

Several subsequent studies have yielded contradictory results, leading to much controversy. Of nine studies included in a recent meta-analysis,¹⁵ five indicated a protective effect of previous use of oral contraceptives with relative risks of 0.39 to 0.70.^{5 14 16-18} The remaining four studies showed no difference in the prevalence of rheumatoid arthritis in relation to previous oral contraceptive use.¹⁹⁻²² The combined total showed an adjusted odds ratio of 0.73 (95% CI 0.61 to 0.85).

These discrepancies, which seemed to distinguish between studies from Europe (positive) and from America (negative),²³ may be explained on the basis of patient selection: pooled odds ratio for hospital based studies was 0.49 (95% CI 0.39 to 0.63) and for population based studies 0.95 (95% CI 0.78 to 1.16).¹⁵

This suggests that oral contraceptives protect only against severe cases of the disease—that is, those usually referred to hospital. This interpretation has been reinforced by the results of a study in which the patients were stratified according to disease progression: a protective effect for oral contraceptives could only be shown for severe cases of rheumatoid arthritis (van Zeben J, personal communication). The same correlation could be seen in the report of preliminary results of an American study showing, for the first time, a protective effect: this is a hospital based study.²⁴

A protective effect of postmenopausal replacement oestrogens has also been suggested,^{5 25} but results are highly contradictory.^{24 26} Finally, a recent study also found that early menarche was associated with a decreased risk of rheumatoid arthritis of about 40% compared with a menarche at an average age.⁷ An additive effect of nulliparity and non-oral contraceptive use has been shown with a fourfold relative risk for the women associating these features.

Some investigators suggest that the protective

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effect of pregnancy as well as oral contraceptive use might be due to the immunomodulating effects of female hormones.^{6,7} The immunological effects of sex hormones are well known and are responsible, at least in part, for changes in disease activity during the menstrual cycle and the amelioration of the disease during pregnancy. Pregnancy is also associated with the production of high concentrations of other immunodepressant substances, such as the pregnancy associated α_2 glycoprotein.²⁷ A long-standing effect, supplying protection against the onset of disease decades after the event, is difficult to explain, however.

Recent studies have suggested that pregnancy and previous oral contraceptive use might be related to persistent hormonal changes translated as very small differences in sex hormone binding globulin concentrations.²⁸ The significance of these findings is far from established but does not support a major role for these effects.

One other possible explanation is that pregnancy or oral contraceptive use might be markers of behavioural patterns leading to a reduced risk for the disease owing to changes in risk of exposure to virus or other potential pathogenic factors. This seems rather contradictory, however, given that, in principle, the use of oral contraceptives should associate with sexual activity and avoidance or later onset of pregnancy.²⁹

Heat shock proteins and arthritis

Heat shock proteins have been related to arthritis in a variety of ways, both in animal models and human disease, and have been the subject of a number of recent reviews.³⁰⁻³²

Adjuvant arthritis in rats is induced by the injection of mycobacteria emulsified in oil (complete Freund's adjuvant). Cohen's group showed that an important element of the adjuvant's arthritogenic properties is a heat shock protein of 65 000 molecular weight—hsp 65 kD.³³ The arthritis can be transferred to irradiated rats by one arthritogenic T cell clone, shown to be reactive against both *Mycobacterium tuberculosis* and cartilage proteoglycan.³⁴ Later it was possible to isolate two T cell clones responsive to a small sequence of amino acids in the hsp 65 kD.³⁵ Although one of these clones is able to transfer the arthritis to irradiated naive rats (A2b), the other one (A2c) suppresses the development of arthritis on subsequent exposure to the adjuvant.^{33,36} The treatment of the arthritogenic clone by hydrostatic pressure also rendered it capable of protecting against arthritis.³⁶

More recently it has been shown that treatment of animals with purified mycobacteria hsp 65 obtained by recombinant DNA technology does not induce arthritis but, on the contrary, suppresses its development on subsequent exposure to the complete adjuvant.^{35,37} The same treatment also protects against experimental arthritis induced by streptococcal cell wall³⁸ but not against a lipoidal amine arthritogenic adjuvant, which is claimed to be non-antigenic.³⁷

Thus a 'vaccine' against several models of experimental arthritis had been discovered, opening a new avenue of research in human disease, as one of the most remarkable characteristics of heat shock proteins is that they are highly conserved among different species of bacteria and retain a notable similarity in eukaryotes, including man.³²

Several studies support the idea that the mechanisms involved in the animal model may also be relevant in human disease. The mycobacterial hsp 65 kD, for instance, has a 50% homology with a known human heat shock protein with a molecular weight of 58 000.³⁹ Recently, it has been shown that heat shock proteins, represent the predominant antigens, not only of mycobacteria but also of other micro-organisms related to the induction of reactive arthritis.⁴⁰

T lymphocytes from patients with rheumatoid arthritis show increased responsiveness to a mycobacterial antigen that is cross reactive with cartilage proteoglycans.⁴¹ Mononuclear cells from synovial fluid of patients both with reactive and rheumatoid arthritis have been shown to proliferate after exposure to hsp 65 kD.^{42,43} This response is found in about two thirds of patients with early chronic arthritis and tends to fade in each joint from three years after onset of inflammation.⁴²

Antibodies to mycobacterial hsp 65 kD and human hsp 70 kD have also been found to occur in significantly higher levels in patients with rheumatoid arthritis and ankylosing spondylitis as compared with normal subjects and patients with systemic lupus erythematosus.^{44,45} Antibodies against mycobacterial hsp 65 kD in patients with rheumatoid arthritis have been shown to be cross reactive with the human homologue hsp 58 kD.⁴⁶

The serum of patients with rheumatoid arthritis is able to recognise 70 kD and 20 kD antigens in an extract of synoviocytes,⁴⁷ and studies on rheumatoid synovium have demonstrated considerable amounts of a material cross reactive with mycobacterial hsp 65 kD and human stress protein 72 kD.⁴⁸

For a long time rheumatologists have been interested in the concept that rheumatoid arthritis and other chronic arthritides might be caused by the generation of T cells cross reactive with articular antigens. Recent investigation has put heat shock proteins in the spotlight of the search for the agent responsible for triggering these cells.

Heat shock proteins, sex hormones, and development

The physiological roles of heat shock proteins are complex,^{32,49,50} but some of them are known already to be related closely to genes and receptors of considerable relevance to the pathogenesis of rheumatic diseases and the activity of sex hormones.

The human hsp 70 kD gene lies within the major histocompatibility complex,⁵¹ and a member of this family has recently been implicated in mechanisms of antigen presentation.⁵² Heat shock protein 90 kD has been identified as

a component of the non-transformed 8S/9S receptors of steroid hormones, including progesterone, oestrogen, androgen, and glucocorticoid, in several animal species and in man.⁵³⁻⁵⁷ It also exists in considerable amounts in a free state, mainly in the cytoplasm, where it constitutes a sizable proportion of 0.1 to 2% of the total proteins.^{54, 55} Recently, it was shown that the synthesis of the 90 kD protein is markedly increased in T cells during mitogen stimulation⁵⁸ and its association with tyrosine kinases suggests a general role in cell activation.⁵⁹ Interestingly, Kubo *et al* reported the production of both hsp 70 and 90 kD by chondrocytes of patients with osteoarthritis.⁶⁰

Several investigators have shown that the production of some of these heat shock proteins, including the 90 kD and 110 kD types, is dependent on sex hormones in a tissue specific way and their concentrations are markedly raised by acute and chronic stimulation with oestrogen, progesterone, or both.^{61, 62} Others have also shown that the stimulation of granulosa cells by gonadotropins to produce large amounts of progesterone is accompanied by the production of large quantities of hsp 90 kD, reinforcing the concept that this protein has an important role in the regulation of steroid hormone action in target tissues.⁶³

The relation between sex hormones and heat shock proteins has been given little attention in rheumatological publications.

Recent research showed important peculiarities in heat shock response during prenatal development. In addition to a refractory period, during which heat shock does not induce a typical response, specific types of heat shock protein are expressed spontaneously by fetal tissues in the absence of stress. These belong mainly to the 70 kD family and have been found not only in *Drosophila* species,^{64, 65} *Xenopus laevis*,⁶⁶ and yeasts⁶⁷ but also in mammals.^{68, 69}

Rheumatoid arthritis, sex hormones, and heat-shock proteins: hypothesis

Pregnancy represents a powerful and prolonged oestrogenic, progestagenic, and gonadotropinic challenge. The concentrations of oestriol are, by the third trimester, about 1000 times those seen during a normal menstrual cycle.⁷⁰ During hormonal contraception women are also exposed to higher than normal concentrations of oestrogens.

It is conceivable, taking into account what has already been proved in animals, that these conditions may elicit the production of higher amounts of endogenous heat shock proteins. Pregnancy and oral contraception may exert their protective effect against rheumatoid arthritis by inducing high levels of heat shock proteins responsible for the induction of a state of immunotolerance to subsequent exposure to the actual triggering agent of rheumatoid arthritis. An ideal sequence and timing of the events might be essential for a protective effect. Alternatively, the pregnant woman might be exposed to specific types of heat shock protein produced by the fetus in higher concentrations than seen in adults. Another possibility is that fetal heat

shock proteins are produced which are unique to developing tissues and therefore unknown to the mature immune system of the mother.

The proposed mechanism satisfies most of the conditions known to favour the occurrence and persistence of immunological tolerance: an underlying state of immunosuppression, similarity with antigens of the host, and predominant involvement of T cells, in which tolerance can be induced by smaller amounts of antigen and retained longer.⁷¹

Such a mechanism would also explain the puzzling lack of a dose-response effect in the relation between pregnancy or oral contraceptive use and the incidence of rheumatoid arthritis, as well as the protective effect of an earlier menarche⁷ and a younger age at first pregnancy.⁶

A possible mechanism for the induction of immunotolerance would be the predominant stimulation of a suppressor T cell clone, similar to the A2c clone described in relation to adjuvant induced arthritis. Anti-idiotypic antibodies would represent another possibility.

The high degree of homology shared by heat shock proteins from humans and from different species of micro-organism has been suggested to underlie the chronic maintenance of immunoreactivity in patients with rheumatoid arthritis.³⁰ The same mechanism could be invoked to explain the persistence of immunotolerance for decades after pregnancy. The expression of human heat shock proteins at the cell surface, known to occur under several stress conditions,⁷² may have the same effect.

Clearly, there are several gaps in this hypothesis. Studies on the relation between rheumatoid arthritis and hsp 90 kD or sex hormones and hsp 65 kD are necessary as, to our knowledge, there are no such reports, negative or otherwise. One single study shows that antibodies against hsp 90 kD have been found in patients with systemic lupus erythematosus, but not in a small group of rheumatoid patients used as controls.⁷³ Conversely, proteins antigenically related to hsp 90 kD have been shown in the surface of macrophage-like cells in the synovial fluid of patients with rheumatoid arthritis.⁷²

No answers to the simple questions, such as the possible protective effect of pregnancy and oestrogenic or progestagenic challenges against subsequent induction of experimental arthritis have, apparently, been published. The heat shock protein responses to sex hormones and pregnancy certainly deserve further investigation and eventual persistent changes in immunoreactivity after contraception and pregnancy also require further analysis.

There is a long way to go before clarifying all the possibilities, but the data already gathered justify the need for greater research, and the hope of a 'vaccine' against rheumatoid arthritis is certainly an exciting possibility.

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