Annals of the Rheumatic Diseases

Leaders

Do sex hormones modulate the synovial macrophages in rheumatoid arthritis?

It is becoming apparent that various host influences are important determinants in the development of autoimmune diseases, including rheumatoid arthritis (RA).

The organism responds to inflammatory stimula with coordinated series of adaptive responses involving the immune, nervous, and endocrine systems.

Although immunogenetics may dominate the susceptibility to develop the disease, the most powerful additional factor recognised in the host is the sex of the patient.

Recent epidemiological, clinical, and laboratory evidence has suggested that sex hormones play a central part in the immune response and the immune mediated pathological conditions.[1]

Overall, women have greater humoral and cellular immune responses and therefore may be more susceptible than men to autoimmune diseases, including RA. Women have higher immunoglobulin concentrations than men and produce greater antibody responses to various microorganisms after immunisation. Cell mediated immune response is also stronger in women as shown by a more efficient rejection of allografts and relative resistance to immunotolerance.[2]

On the other hand, women have greater plasma corticotropin (ACTH) response to ovine corticotropin release hormone (CRH) and more prolonged increases in cortisol values, thus indicating CRH neuron activation by oestradiol.[1] Conversely, androgens inhibit the hypothalamic-pituitary-adrenal axis (HPA) supporting the complex interactions existing between the immune response and neuroendocrine system.

In synthesis, evidence suggests that physiological concentrations of oestrogens stimulate while male hormones suppress the immune response.

As the distinct female preponderance in autoimmune diseases exists mainly during the reproductive ages, sex hormone concentrations and metabolism have been evaluated in the affected patients (that is, RA, systemic lupus erythematosus (SLE)) and have often been found to be changed.

In particular, low gonadal and adrenal androgens (testosterone (Tes)/dihydrotestosterone (DHT) and dehydroepiandrosterone sulphate (DHEAS), respectively), as well as reduced androgens/oestrogens ratio, have been detected in male and female RA patients, suggesting a reduction of the related immunosuppressive effects.[3–6]

An important unknown factor in the pathogenesis of RA is the reason why immunological mediated inflammation begins and develops within joints. A central role in understanding RA pathogenesis lies in the comprehension of arthrotopism of antigens and inflammatory cells for joints and in learning what specific receptors and chemotactic gradients are active in focusing the immune mediated inflammation within joints.

Undoubtedly the synovial tissue in RA can be regarded as the 'target tissue' in which the sexual dimorphism in immune response to relevant trigger antigens is present.

In contrast with the cell centred models of RA pathogenesis in which proliferation of antigen specific T cells determines various manifestations of RA, synovitis such as B cell stimulation, leucocyte infiltration, and cytokine synthesis, a key role for synovial macrophages is outlined in which by paracrine and autocrine mechanisms the inflammatory changes in the RA synovium are maintained.[7 8]

Furthermore, proinflammatory cytokines (for example, tumour necrosis factor α (TNFα), interleukin 1 (IL1), interleukin 6) released from the inflamed synovial tissue, reach the brain where they trigger various neuroendocrine counter-regulatory mechanisms and account for most of the HPA stimulation, including the increased release of neuropeptides such as CRH.[1 2] In the light of the above, a question arises: Is there evidence that sex hormones might modulate synovial macrophages in RA?

To validate the possible sex hormone modulation of synovial macrophages, functional receptors for androgens and oestrogens must be present on these cells.

Actually, androgen and oestrogen receptors have been described in both normal and RA synovial macrophages using double immunostaining techniques of synovial tissue cryosections. Biochemical characterisation confirmed the evidence of functional type I and II androgen and oestrogen binding sites in both cytosol and nuclear fractions of cultured normal and RA macrophages.[9 10] Interestingly, both receptor contents and binding affinities of these cells were comparable to those previously reported in other well established sex hormone responsive cells and tissues.

Furthermore, specific messenger ribonucleic acids (mRNA) for sex hormone receptors, encoding for a sequence of the DNA binding domain of the receptor protein, have

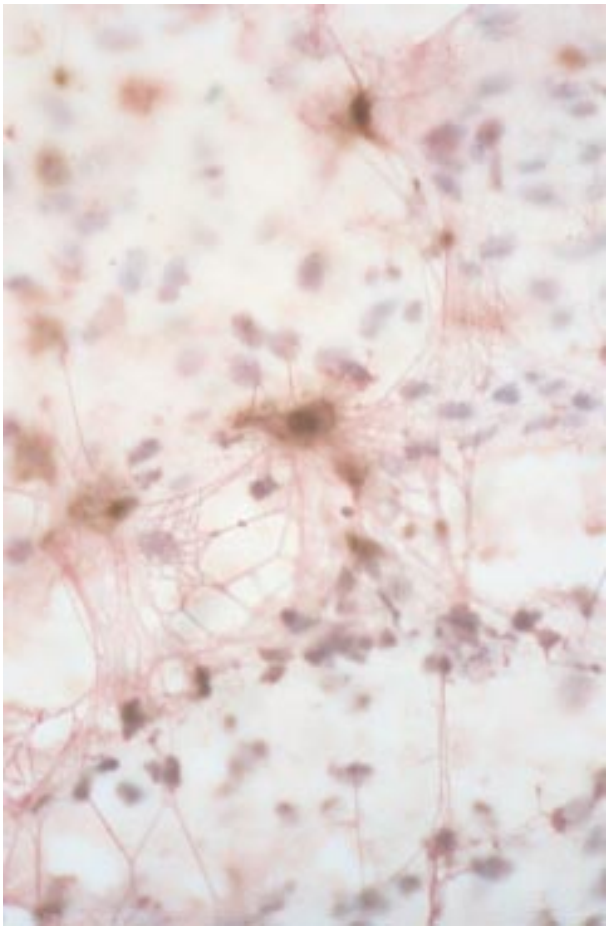

Figure 1 Activated synovial macrophages after double immunostaining with the monoclonal antibodies directed toward the androgen receptor and the phagocytic cell antigen (Ber-MAC3) in the lining of the rheumatoid synovial tissue. A strong nuclear reaction for the androgen receptor (brown) and diffuse staining for the antigens of activated macrophage including the branched cytoplasmic extensions (Ber-MAC3 - red) are present (cryosection, original magnification × 200).

recently been shown by reverse transcriptase-polymerase chain reaction in the same cells.[11]

Therefore, these results might correlate with the modulatory effects envisaged for sex hormones on synovial macrophages and might support their suggested implication in the pathogenesis of RA. Figure 1 shows a cryosection of RA synovial macrophages immunostained with both the anti-androgen receptor monoclonal antibody and the anti-macrophage antigen antibody Ber-MAC3.

Another question is: What might be the effects of sex hormone on cultured monocyte/macrophages? The 'cytokine and proto-oncogene networks'.

Low concentrations of gonadal and adrenal androgens (mean (SEM)) (Tes = 17 (10) nmol/l RA fluids *v* 40 (15) nmol/l non- RA fluids, DHEAS = 900 (500) nmol/l RA fluids *v* 1750 (750) nmol/l non- RA fluids, $p < 0.05$), and increased concentrations of more feminine hydroxylated oestrogen metabolites (16α OHE_1 + 4OHE2 = 211 ng/ml range 8-877 ng/ml RA fluids *v* 61 ng/ml range 8-186 ng/ml non- RA fluids) have also been found in male and female RA synovial fluids (L Castagnetta, personal communication), supporting the complex relation between synovial macrophages and sex hormones at the level of the synovial RA tissue and fluid.[12 13]

Physiological serum concentrations of androgen and oestrogen have been found, respectively, to inhibit and potentiate the basal concentrations of IL1β produced by cultured monocyte/macrophages after their activation.[14–16] In addition, human synovial macrophages have been found capable of metabolising Tes to the more bioactive metabolite DHT.[14]

Interestingly, the effects of oestrogens on IL1 synthesis by macrophages seem dose dependent and a negative relation has been found between IL1 mRNA concentrations and oestrogen concentrations in human peripheral monocytes (precursors of tissue macrophages) and pelvic macrophages.[16] Thus, low oestrogen concentrations (physiological, 10^{-8} M) stimulate both IL1 mRNA values and IL1 protein synthesis, whereas higher concentrations (pharmacological, 10^{-6} to 10^{-5} M) are inhibitory, suggesting a biphasic response. Similar results have been obtained when TNFα mRNA concentrations were evaluated on cultured synovial macrophages (F Di Giovine, personal communication).

This finding might explain some of the influences exerted by exogenous and endogenous oestrogens on clinical aspects of RA, including menstrual fluctuations of RA symptoms. In addition, the higher incidence of RA in women as well as the improvement of the disease during pregnancy and the variable effect of oral contraceptives might be related to dose dependent oestrogen immune modulation of the cytokine synthesis.[17]

However, together with the 'cytokine network' regulating both the macrophage and T cell dependent pathways, a 'proto-oncogene network' may be a major T cell independent pathway that is activated in RA.

Generally, oestrogens seem to prevent apoptosis, whereas androgens seem to induce it. Therefore, low concentrations of androgens as seen in serum samples and synovial fluids of patients affected by RA should, at least partially, determine both reduced synoviocyte apoptosis and synovial tissue hyperplasia.[18]

Synovial macrophages are largely represented on the RA synovial lining (70-90%) and they express most of the proto-oncogene markers of apoptosis, cell growth, and activation described on the hyperplastic RA synovium.[19] A recent study suggested that activation induced macrophage apoptosis might serve to restrict the destructive potential of inflammatory macrophages.[20]

We recently evaluated the c-myc proto-oncogene expression on cytocentrifuge preparations obtained from primary cultures of RA synovial macrophages stimulated with different E2 concentrations: from physiological (10^{-8} M) to pharmacological (10^{-5} M) (unpublished data).

C-myc expression was detected by immunostaining on cytocentrifuge preparations five to 18 hours after stimulation with different E2 concentrations. Intense staining for c-myc was seen after five hours on cytocentrifuge preparations treated with E2 10^{-8} M in absence of LPS (negative at 18 hours).

No staining was seen after E2 10^{-5} M and in presence of LPS, for both concentrations. No staining was found on untreated synovial macrophages.

As c-myc could be detected in about 70-80% of proliferating RA synovial lining cells, and as it is an early proto-oncogene marker of cell growth, we suggest that the observed increased expression of c-myc on synovial RA macrophages treated with physiological E2 concentrations (10^{-8} M) should be mainly related, in our case, to the E2 mediated mitogenic stimulation and prevention of apoptosis.

In fact, oncogenes that encode nuclear proteins (for example, c-myc) have an immortalising activity and prevent cells from senescence, probably correlating with the greater longevity of women.[21]

Experimental studies have previously shown that oestrogen induces the expression of c-fos and c-myc proto-oncogenes in rat uterus.[22] On the contrary,

androgens, among steroids capable of inducing apoptosis, should be considered a biological means of therapy that might improve treatment of autoimmune diseases including RA.

Might there be a therapeutical significance for the sex hormone modulation of synovial RA macrophages? The androgen treatment.

Through secretion of cytokines (IL1, TNFα) synovial RA macrophages can stimulate fibroblast proliferation and activity, which can lead to further cytokine, collagenase, and prostaglandin release, and can also increase HLA-DR expression on synovial cells and can activate T and B cells. Macrophage cytokines also increase the ability of circulating leucocytes to attach and to pass through high endothelial cells of postcapillary venules, thus contributing to induction of new blood vessel formation in RA synovial tissue and finally improving osteoclastic bone resorption.[23] Furthermore, joint destruction has been shown mediated by proliferating RA synovial lining cells attached to cartilage and bone.[24]

Taken together, these findings support the concept that, while the earliest events in the pathogenesis of RA may be dependent upon both T lymphocytes and macrophages, macrophages and fibroblasts are essential for the perpetuation of synovitis and articular damage.[24 25]

A great amount of evidence shows that the number of infiltrating synovial macrophages correlate with the degree of articular destruction in RA.[25 26]

Testing the effects of intraarticular testosterone and DHT on cartilage breakdown and inflammation in animal models of RA, a significant inhibitory activity on synovial hyperplasia and cartilage erosion was found in various studies.[27 28] In addition, in the antigen induced arthritis mouse model, the reduction of extent and severity of synovial hyperplasia for Tes dosed intraarticularly was found to be significantly higher than for intraarticular dexamethasone.[27]

In the light of this evidence, some clinical studies evaluated the role of systemic administration of Tes in RA patients of both sexes and beneficial therapeutic effects were found, including a significant reduction of swollen joint count when compared with placebo treated RA patients.[29–31]

A recent finding seems to further support the possible therapeutic androgen mediated influence on some RA mechanisms in cyclosporin (CSA) treated RA patients.[32]

A constant dose dependent side effect in CSA treated patients is the appearance of hypertrichosis, which occurs in both sexes and suggests an androgenising activity. To determine the CSA influence on peripheral androgen metabolism, we evaluated in RA patients (mean (SD) age 48 (5) years) treated with low dose CSA during a period of 12 months, plasma concentrations of Tes and of 5α-androstane-3α,17β-diol glucuronide (Adiol-G), an important peripheral Tes metabolite. Furthermore, the metabolism of physiological concentrations of Tes (10^{-8} M) was evaluated in primary cultures of RA synovial macrophages in presence of CSA concentrations close to the pharmacological immunosuppressive doses (100-500 ng/ml). At the final time of observation (12 months) a significant increase of the mean plasma Adiol-G concentration was observed in patients of both sexes. Results from in vitro experiments of Tes metabolism by cultured synovial macrophages showed, at 24 and 48 hours in the presence of CSA, a significantly greater formation of DHT and increased amounts of other Tes metabolites when compared with untreated controls. The appearance of a dose related hypertrichosis and the increase of the plasma androgen metabolites (for example, Adiol-G) in CSA treated RA patients, as well as the hormonal metabolic effects on cultured synovial macrophages, should be regarded as possible markers of the CSA influence on peripheral androgen metabolism at the level of target cells.[32]

In conclusion, the question: Do sex hormones modulate synovial macrophages in rheumatoid arthritis? should be considered. The answer is yes.

Sex hormones, among other factors (for example, infectious, genetic, stress related) may influence the susceptibility to develop RA. The synovial cells, in particular synovial macrophages, should be considered the major sex hormone responsive 'target cells'.

Furthermore, considering that physiological concentrations of oestrogens stimulate while male hormones suppress the immune response and in the light of the changed sex hormone concentrations observed in RA patients, additional innovative biological treatments of RA for combination therapies might include the intraarticular administration of androgens or androgen mediated immunosuppressive drugs or even antioestrogen compounds such as tamoxifen.

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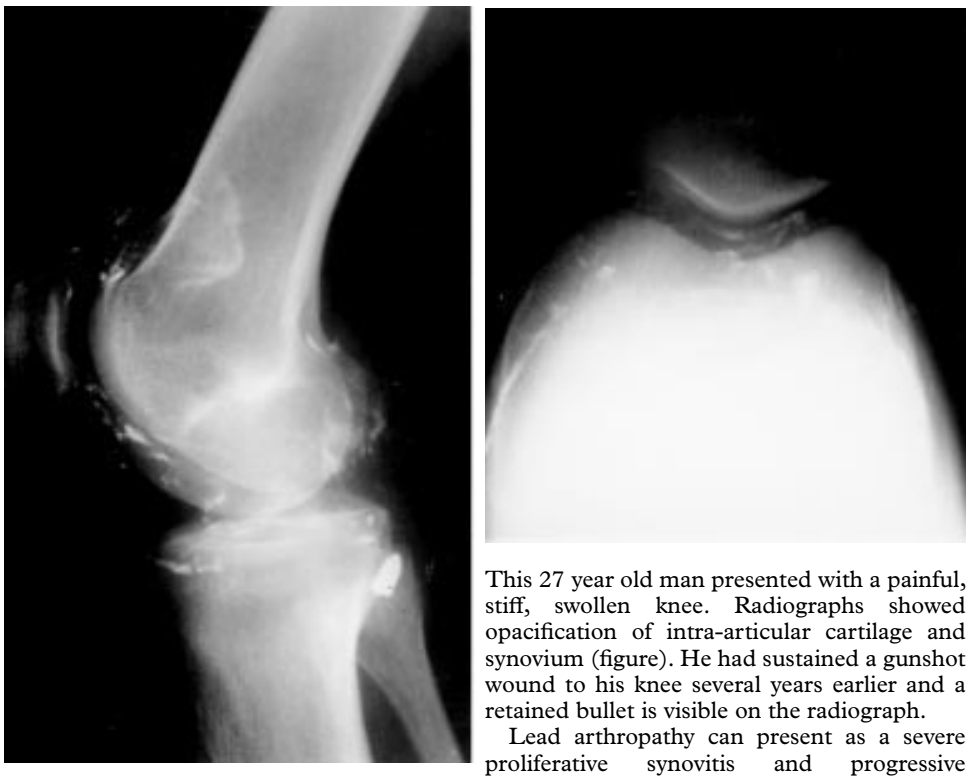
1 Chrousos GP. The hypothalamic-pituitary-adrenal axis and immune mediated inflammation. N Engl J Med 1995;332:1351-62.
2 Wilder RL. Neuroendocrine-immune system interactions and autoimmunity. Annu Rev Immunol 1995;13:307-38.
3 Cutolo M, Balleari E, Giusti M, Monachesi M, Accardo S. Sex hormone status of male patients with rheumatoid arthritis: evidence of low serum concentrations of testosterone at baseline and after human chorionic gonadotropin stimulation. Arthritis Rheum 1988;31:1314-7.
4 Cutolo M, Balleari E, Giusti M, Monachesi M, Accardo S. Sex hormone status in women suffering from rheumatoid arthritis. J Rheumatol 1986;15:561-5.
5 Lahita RG, Bradlow HL, Kunkel HG, Fishman J. Increased 16 alpha-hydroxylation of estradiol in systemic lupus erythematosus. J Clin Endocrinol Metab 1981;53:174-8.
6 Masi AT, Josipovic DB, Jefferson WE. Low adrenal androgenic-anabolic steroids in women with rheumatoid arthritis (RA): gas-liquid chromatographic (GLC) studies of RA patients and matched normal control women indicating decreased 11-deoxy-17-ketosteroid excretion. Semin Arthritis Rheum 1984;14:1-23.
7 Cutolo M, Sulli A, Barone A, Seriolo B, Accardo S. Macrophages, synovial tissue and rheumatoid arthritis. Clin Exp Rheumatol 1993;11:331-9.
8 Athanasou NA. Synovial macrophages. Ann Rheum Dis 1995;54:392-4.
9 Cutolo M, Accardo S, Villaggio B, Clerico P, Indiveri F, Carruba G, *et al.* Evidence for the presence of androgen receptors in the synovial tissue of rheumatoid arthritis patients and healthy controls. Arthritis Rheum 1992; 35:1007-15.
10 Cutolo M, Accardo S, Villaggio B, Clerico P, Bagnasco M, Coviello DA, *et al.* Presence of estrogen binding sites in synovial macrophages and CD8+CD29+ CD45RO+ T lymphocytes in normal and rheumatoid synovia. Arthritis Rheum 1993;36:1087-97.
11 Cutolo M, Accardo S, Villaggio B, Barone A, Sulli A, Coviello DA, *et al.* Androgen and estrogen receptors are present in primary cultures of human synovial macrophages. J Clin Endocrinol Metab 1996;81:820-7.
12 Cutolo M, Sulli A, Seriolo B, Accardo S, Masi AT. Estrogens, the immune response and autoimmunity. Clin Exp Rheumatol 1995;13:217-26.
13 Masi AT, Da Silva JA, Cutolo M. Perturbations of hypothalamic-pituitary-gonadal (HPG) axis and adrenal androgen (AA) functions in rheumatoid arthritis. In: Chikanza IC, ed. Neuroendocrine immune mechanisms of rheumatic disease. Ballieres Clin Rheumatol 1996;2:295-332.
14 Cutolo M, Accardo S, Villaggio B, Balleari E, Cannella S, Faruggio R, *et al.* Cultured synovial macrophages from rheumatoid arthritis synovium metabolize testosterone and exhibit inhibition of IL-1β production. Arthritis Rheum 1993;36 (suppl):S158.
15 Hu SK, Mitcho YL, Rath N. Effect of estradiol on interleukin 1 synthesis by macrophages. Int J Immunopharmacol 1988;10:247-52.
16 Polan ML, Loukides J, Nelson P, Carding S, Diamond M, Walsh A, *et al.* Progesterone and estradiol modulate interleukin-1β messenger ribonucleic acid levels in cultured human peripheral monocytes. J Clin Endocrinol Metab 1989;69:1200-6.
17 Bijlsma JW, Van Den Brink HR. Estrogen and rheumatoid arthritis. Am J Reprod Immunol 1992;28:231-4.
18 Cutolo M, Sulli A, Barone A, Seriolo B, Accardo S. Sex hormone, proto-oncogene expression and apoptosis: their effects on rheumatoid synovial tissue. Clin Exp Rheumatol 1996;14:87-94.
19 Firestein GS, Yeo M, Zvaifler NJ. Apoptosis in rheumatoid arthritis synovium. J Clin Invest 1995;96:1631-896.
20 Munn DH, Beall AC, Song D, Wrenn RW, Throckmorton C. Activation-induced apoptosis in human macrophages: developmental regulation of a novel cell death pathway by macrophage colony-stimulating factor and interferon γ. J Exp Med 1995;181:127-36.
21 Sorrentino V, Drozdoff V, McKinney MD. Potentiation of growth factor activity by exogenous *c-myc* expression. Proc Natl Acad Sci USA 1986;83:8167-71.

22 Weisz A, Bresciani F. Estrogen induces expression of *c-fos* and *c-myc* proto-oncogens in rat uterus. Mol Endocrinol 1988;2:816-24.
23 Fujikawa Y, Sabokbar A, Neale S, Athanasou NA. Human osteoclast formation and bone resorption by monocytes and synovial macrophages in rheumatoid arthritis. Ann Rheum Dis 1996;55:816-22.
24 Geiler T, Kriegsmann J, Keyszer GM, Gay RE, Gay S. A new model for rheumatoid arthritis generated by engraftment of rheumatoid synovial tissue and normal human cartilage into scid mice. Arthritis Rheum 1994;37:1664-71.
25 Zvaifler NJ. Macrophages and the synovial lining. Scand J Rheumatol 1995;24 (S101): 67-75.
26 Mulherin D, Fitzgerald O, Bresnihan B. Synovial tissue macrophage populations and articular damage in rheumatoid arthritis. Arthritis Rheum 1996;39:115-24.
27 Steward A, Bayley DL. Effects of androgens in models of rheumatoid arthritis. Agents Actions 1992;35:268-72.
28 Da Silva JAP, Larbre JP, Seed M, Cutolo M, Villaggio B, Scott DL, *et al.* Sex differences in inflammation induced cartilage damage in rodents. J Rheumatol 1994;21:330-7.
29 Cutolo M, Balleari E, Giusti M, Intra E, Accardo S. Androgen replacement therapy in male patients with rheumatoid arthritis. Arthritis Rheum 1991; 34:1-5.
30 Booij A, Biewenga-Booij CM, Huber-Bruning O, Cornelius C, Jacobs JWG, Bijlsma JWJ. Androgens as adjuvant treatment in postmenopausal female patients with rheumatoid arthritis. Ann Rheum Dis 1996;55:811-5.
31 Cutolo M, Castagnetta L. Immunomodulatory mechanisms mediated by sex hormones in rheumatoid arthritis. Ann N Y Acad Sci 1996;784:534-41.
32 Cutolo M, Giusti M, Villaggio B, Barone A, Sulli A, Accardo S, *et al.* Testosterone metabolism and cyclosporin A treatment in rheumatoid arthritis. Br J Rheumatol 1997;36:433-9.

Unusual but memorable

Series editor: Gary D Wright



This 27 year old man presented with a painful, stiff, swollen knee. Radiographs showed opacification of intra-articular cartilage and synovium (figure). He had sustained a gunshot wound to his knee several years earlier and a retained bullet is visible on the radiograph.

Lead arthropathy can present as a severe proliferative synovitis and progressive destructive arthritis. The earliest radiographic finding is fine punctate deposition of radio-opaque lead on articular cartilage similar to chondrocalcinosis but with increased density. Discrete lead speckling of hypertrophied synovium follows and eventually the articular cartilage and joint capsule may be completely outlined. Histopathological studies have confirmed synovial hypertrophy, diffuse chronic inflammation, and fibrosis with areas of haemosiderin deposition and calcification.[1] Several interactive pathological processes may be involved, including mechanical trauma and chemical destruction.[2]

1 Sclafani SJA, Vuletin JC, Twersky J. Lead arthropathy: arthritis caused by retained intra-articular bullets. Radiology 1985; 156:299-302.
2 Leonard MH. The solution of lead by synovial fluid. Clin Orthop 1969;64:255-61.

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