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Musculo-skeletal joint inflammatory diseases - New vistas from animal model studies – An overview

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Introduction

The term 'Arthritis' refers to all categories of musculo-skeletal joint inflammatory diseases. The different profiles of arthritic manifestations include rheumatoid arthritis, ankylosing spondylitis, osteoarthritis, polyarthritis, systemic lupus erythematosus (SLE), juvenile arthritis, gout and psoriatic arthritis etc. All these inflammatory diseases are characterized by chronicity and non-reversible nature of the pathogenesis. All types of arthritis show some common pathological changes like synovial hypertrophy, microvascular abnormalities, leucocytes infiltration, neo-vascularisation etc. Though animal model studies on human diseases may not fully mimic the real in vivo intricacies of human diseases, certain aspects of such studies and comparison of the findings to real situations may provide insights into the etiological, pathological and therapeutic profiles.

Keywords: Arthritogenesis, cytokines, tumour necrosis factor, calcium influx

Musculo-skeletal architecture

In the musculo skeletal regions, polysaccharides and protein units behave as large polyanions and cations to form the ground substances. The proteoglycans afford the visco-elastic properties of joints. The polysaccharide chains of proteoglycans are made up of repeating disaccharide units viz., the glucosamines or galactosamines (Stryer, 1981). In the pathogenesis of connective tissue, the catalytic/catabolic reactions preponderate while the anabolic functions concurrently are arrested.

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Autoimmune diseases

Rheumatoid arthritis (RA) is the most common in autoimmune/rheumatic disorders. It is a chronic non-supparactive inflammatory disease mainly affecting peripheral synovial joints, usually exhibiting a symmetrical pattern and prolonged course with exacerbation and remission. The onset of the RA may be at any age but most frequent from 35 to 50 years of age. Women are affected around three times more often than men. The joints in the early phase exhibit readness, pain, heat and localized swelling. Any of the small joints may be affected in this RA.

Ankylosing spondylitis (AS) is one of the systemic diseases predominantly of young men, involving the lumbar spine and sacro-iliac joints. This disease is characterized by the inflammation at the attachment of ligaments to bones mainly consisting of lymphocytes infiltration. The spine becomes fused to form a rigid structure mainly affecting hip, knee, ankle and shoulder.

Systemic Lupus Erythematosus (SLE) is a polyarticular joint disease, occasionally progressing to deforming chronic disease. Wrists, knees and fingers are mostly affected. Multiple tissues including skin, mucosa, kidney, brain and cardio vascular systems are involved in the pathogenecity.

Osteoarthritis (OA) is a non-inflammatory disorder of diarthridial joints characterized by progressive loss of cartilage, sclerosis of underlying bone and proliferation of bone and cartilage at the joint margin.

Juvenile arthritis (JA) refers to Juvenile rheumatoid arthritis (JRA) as well as Juvenile chronic arthritis (JCA) or juvenile idiopathic arthritis (JIA). The peak onset of JA is 1-3 yrs and in teenagers. Rarely, below 6 months old also can get. Females are more prone to than males. The knee is the most commonly affected joint. The ankle stands next in frequency. Other joints are less frequently affected. JA may be rheumatoid factor positive or negative. The clinical manifestations include lymphadenopathy, hepatosplenomegaly, microcytic and hypochronic anemia. The cause for JA is unknown. However the immunological basis and immunogenetic (HLA) associations cannot be ruled out (Glass and Giannini, 1999). Infections of environmental pathogenesis also seem to be attributed for JA pathogenesis.

Epidemiology (Incidence)

The centre for disease control, USA gave a detailed statistics of arthritic disease prevalence in the country. Accordingly in USA, 9500 people die and 7, 50, 000 individuals are hospitalized every year. About 8 million people will be expected to suffer in future and 30 lakhs of children to exhibit the symptoms in their childhood. 86 billion dollars are being spent per annum. Similarly, in UK about 8.5 million individuals per year suffer from osteoarthritis (moderate to severe) while approximately 3, 87, 000 suffer from RA. The government expenditure there goes to 5.7 billion pounds annually. The disease has been extrapolated to other European and Asian countries based on US statistics, which will be astounding in billions of population in the world. The above magnitude and dimension of these chronic ailments throughout the world remain to be a cause for concern and warrant new lines of research investigations towards their alleviation.

Biochemical factors

Since arthritis involves damage to tissues, the contribution of various metabolites and the activities of such enzymes as hydrolases and transaminases (GOT and GPT) have been implicated. Towards this, enzymes play a major role in either synthesizing or dispensing with the metabolic substrates. In rheumatology, it is of interest to derive insights regarding the changes in metabolites and enzymes and to understand whether the changes are generalized one involving all tissues or specific to musculo-skeletal tissues of joint alone. Animal model studies have revealed that adjuvant arthritis is associated with growth failure, hypermetabolism and accelerated proteolysis (Lopez *et al*., 1999). The C-reactive protein has been demonstrated to significantly increase consequent to CFA and IFA induced arthritogenesis in wistar rats *Rattus norvegicus* and to decrease in drug treated rats (Table 1). The abundance of lysosomal enzymes in tissues like spleen and liver, the abnormal fragility of the lysosomal enzymes and their discharge and/or seepage have been implicated in a number of pathological phenomena involving inflammatory reactions including rheumatoid arthritis (Dingle, 1961, Geetha and Varalakshmi, 1999).

Role of Uric acid/Urate

Purine degradation is responsible for uric acid production (Aspliz, 1996). The enhanced content of uric acid is a diagnostic parameter in joint inflammation. The uric acid production is also correlated to deficiency of renal function and neurological dysfunction (King and Nicholas, 1977; Curto *et al*., 1998). The renal dysfunction leads to decreased excretion of uric acid (Hosoya, 1996). It was also reported that tissue anaerobic condition represents a predisposing factor for the acceleration of purine nucleotide degradation (Yamanaka *et al*., 1992). Impaired renal function in gout has been reported and that the defect in uric acid excretion is more a consequential effect in inflammatory conditions than uric acid production (Trang et al., 1995; Curto et al., 1998). The uric acid precipitation in medulla of kidney also correlated with the decreased uric acid supersaturation in the urine (Ichida 1996). The participation of uric acid in cell mediated reactions has also been delineated. For instance, the experimental oxonate induced hyperuricemia in rats seemed to have influenced the secondary cell mediated reactions without affecting the acute inflammatory process (Lussier et al., 1978). The infection of *Mycobacterium tuberculosis* with concurrent accumulation of monosodium urate crystals has been demonstrated by Lorenzo *et al*., (1997). Weinberger (1995) has revealed that the interactions of monosodium crystals with granulocytes-macrophage colony stimulating factor-activated neutrophils favours the production of IL-1 and results in pro-inflammatory imbalance. He has also revealed that a similar interaction of the urate crystals with TNF, activated neutrophils in favouring the release of IL-1. Moreover, mononuclear cells containing phagocytosed monosodium urate crystals is a regular feature in the synovial fluid of the patients with gout and the interaction of these cells and crystals seem to induce arthritis in gouty patients (Pascual and Jovani, 1995). In gouty arthritis, urate crystals deposition is followed by the catalytic generation of reactive oxygen species (Ghio *et al*., 1994). The crystals thus can induce phagocytosis by leucocytes. They also induce neutral protease synthesis and secretion of arachidonic acid and its metabolism in synoviocytes and macrophages (McCarthy, 1994). The induction and activation of proteases like cathepsin by the antigen-antibody reactions has been reported as early as in 1960, in the cultured tissue monocytes and in the slices of guinea pig lung and human skin (Hayashi and Tokuta, 1960).

Table 1: CRP levels in control and test groups (Wistar rats)

Control	Complete Adjuvant Treated	Complete and Incomplete Adjuvant treated	Diclofenac Sodium Treated
Negative	Negative	20ng/dl	8ng/dl

Tissue pathogenesis

In arthritis, inflammatory mediators exhibit not only suppression of matrix synthesis but also cartilage degradation systematically. Locally produced growth factors and hormones generally regulate the cartilage metabolism (Verschre *et al*., 1996). However, during acute joint reaction, proteoglycans released out from cartilage induce autoimmune responses against cartilage which contribute chronification of inflammation and cartilage degradation (Braver *et al*., 1996). The severity of inflammation and its chronicity are major determinants of the degree of chondrocytes proteoglycans synthesis inhibition as well as their destruction, leading to irreversible joint destruction (Kruijsen *et al*., 1985). Free proteoglycan fragments may also mediate pathological changes in arthritic joints (Boniface *et al*.,

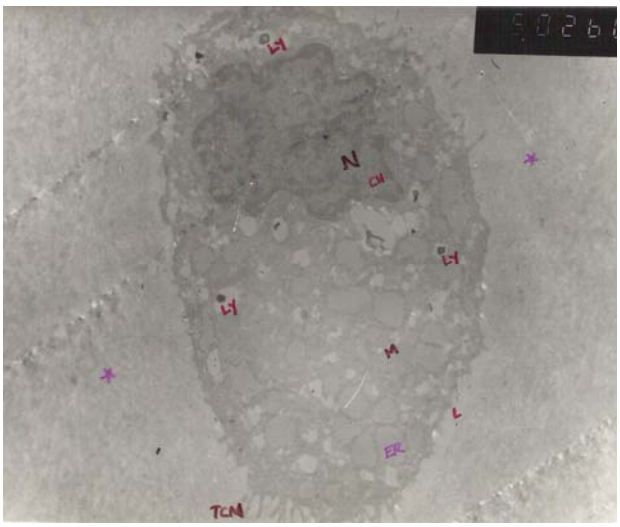

Fig 1. Hypertrophied Chondrocyte cell in CFA induced arthritogenesis in wistar rats (8000 X). *Promident extra cellular matrix, L- Lacuna, TCM – Typical chondrocyte cells membrane, ER – Endoplasmic reticulum (filled with material), Ly – lysosomes, N – Nucleus, M- Mitochondria, CH – Heterochromatin.

1988). Thus the culmination of cartilage damage include loss of functional activity of chondrocytes and loss of compensatory

proliferation of chondrocytes. The chondrocytes destruction was also attributed to hyperplasia of various organelles and golgi complex in particular (Pavlova and Duliapin, 1976; Beesley *et al*., 1992).

As for chondrocytes, their function is dual, that is, they can assume either the catabolic or the anabolic phase of activity. For degenerative changes the failure of chondrocytes proliferation and their death at the articular cartilage have been attributed (Kruijsen *et al*., 1985). However our study illustrated the hypertrophic state of chondrocytes in the adjuvant induced arthritogenesis (Fig 1). In this context, Braver et al., (1996) have opined that the release of proteoglycans may augment the inflammation. Previously Boniface et al., (1988) have also stressed that free proteoglycan fragments may mediate the joint inflammation. In the light of the above studies, the hypertrophic condition of chondrocytes may be taken to indicate the synthesis of proteoglycan mediators to enhance the tissue lesion further.

CFA induced arthritogenesis in wistar rats. The localization of macrophage and T lymphocytes in the joint tissue foci:

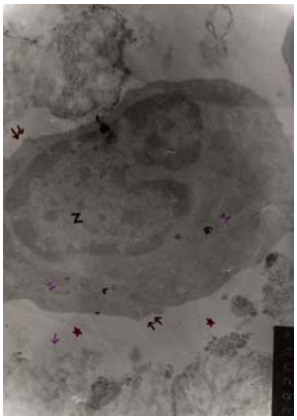

Fig 2

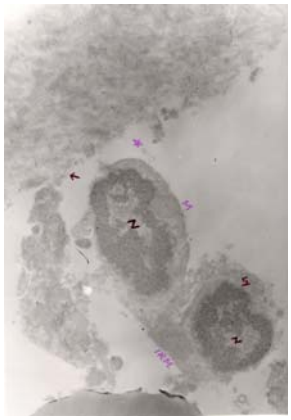

Fig 3

Fig 2. Macrophage (8000x) → Extra cellular matrix, * Digested extra cellular matrix, Macrophage in apposition to Synovial space, G- golgi complex, M- mitochondria, N-nucleus, Ly-Lysosomes.
Fig 3. Cytotoxic T-lymphocytes (8000x) → Extra cellular matrix, N- nucleus, M- Mitochondria, IRM – Irregular shape mitochondria, y-Lysosomes, * Digested extra cellular matrix.

Cytotoxic phenomenon

Cell mediated destructive process is characteristic of joint disorders. CFA induced arthritogenesis in wistar rats *Rattus norvegicus* has demonstrated the involvement of cytotoxic T lymphocytes and the macrophages in the erosion of cartilage surface and near Pannus (Personal observations) (Fig 2 & 3). Besides the above cells plasma, cells have also been identified (Chew *et al*., 1990; Beesely *et al*., 1992). Thus phagocytic cells with their high enzyme content bring about the cartilage degradation in the musculo-skeletal joints (Mohr and Wild, 1977).

Auto-Antibodies to bacterial antigens

Though the disease arthritis is attributed generally to the autoimmune mechanism consequent to breakdown/failure in cognition property of normal immune cells to self antigens, the molecular mimicry in the immune system to the viral and bacterial antigens is mostly attributed for its manifestation (Oldstone, 1990; Albani and Carson, 1996). The endogenous self-peptides derived from the polymorphic regions of HLA in human show homology to certain proteins of infectious agents such as *Escherechia coli, Klebsiella, Pneumonia, Shigella sp., Epstein – Barr virus*, etc. These homologous peptides which constitute the shared epitopes, form the basis for persistence of inflammation in autoimmune diseases in general and arthritic diseases in particular. Regarding auto-antibodies, Jennings (1995) demonstrated elevated IgG level in patients with juvenile rheumatoid arthritis. In his study, the immune complexes revealed that they contained IgM RF, IgGRF and also 40 and 60kD constituents (Fig 4). Subramanian and Ramalingam have revealed the expression of IgG in adjuvants (CFA & IFA) treated rats and absence of rheumatoid factor. These authors agree with the hypothesis of Gays *et al*., (1993) that the pathogenesis of arthritis involves the interaction of soluble and insoluble mediators and Ig complex with neutrophil surface receptors and the activated neutrophils release oxygen free radicals and lysosomal enzymes to bring about the tissue destruction. However, in rheumatoid arthritis other immune cells such as lymphocytes, monocytes, macrophages, neutrophils and to a limited extent mast cells and basophils have all been documented as the sources of inflammation, as they have been primed to the inflammatory regions by angiogenesis.

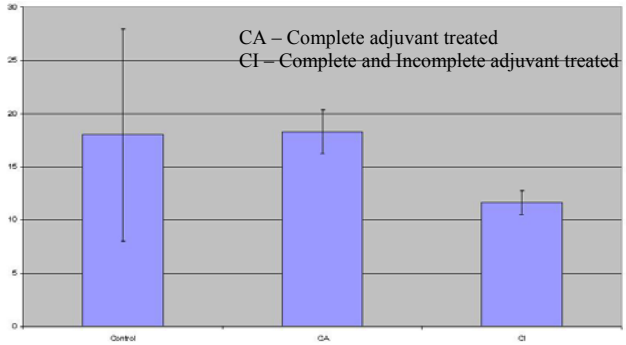

Fig 4. Immunoglobulin - G levels in Control and Test groups

Trace (Metallic) elements in joint inflammation

Since metallic/trace elements like zinc, copper, calcium, selenium, magnesium etc. are implicated to operation of normal biochemical and physiological processes, their role in abnormal arthritogenesis may not be unexpected. The vital roles of trace elements include free radical scavenging, articular tissue formation, immune functions and membrane integrity. Patients with RA have been demonstrated to show deficiencies in regard to copper, zinc, selenium and magnesium. The increase in proinflammatory cytokines IL-1, TMF-α and IL-6 may affect the availability of these trace elements by inducing the production of metal binding proteins metallothionins in the liver and intestine (Kishore 1989; Zoli *et al*., 1998). Enzymes dependent on these trace elements have protective tissue functions against singlet oxygen accumulation as well as

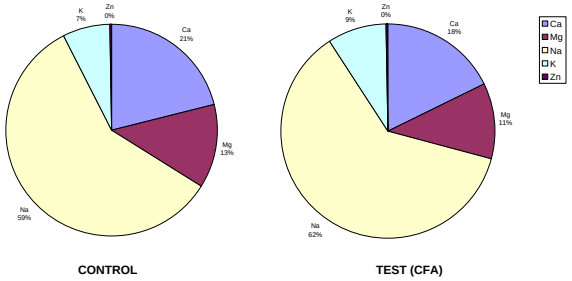

Fig 5. Pie diagram based on percent values of electrolytes between control and test group (CFA)

repair and healing (Sorensen, 1989). Our experiments on adjuvant induced arthritis in wistar rats (CFA) revealed marked variations in the serum level Ca^{2+}, Mg^{2+}, Na^{+}, K^{+} and Zn^{2+} (Fig 5). The hyponatraemia and hyperkalaemia were conspicuous. The Ca^{2+} fell down markedly in the CFA treated wistar rats (743 $\pm$ 11/583 $\pm$ 7 µg/ml). A marked decrease of overall elements in serum viz., Ca^{2+}, Mg^{2+}, Na^{+} and Zn^{2+} occurred. Though the above overall decline in serum may be construed, a generalized reaction of stress and inflammation event, the marked decrease of calcium in serum signifies its entry into tissues/cells. In this context, it is to note that in neurotoxicity the phospholipid hydrolysis and increased Ca^{2+} influx and its consequent accumulation has been attributed to neuronal death (Harvey, 1991). Considering the above and also the calcineurin induced cascade of cell death it may be inferred that the inflammatory and arthro-degenerative changes may be, in addition to immunological factors, mediated by the changes in the extracellular pool of electrolytes especially the Calcium. Conversely Ca^{2+} dependent mammalian phospholipase A_2 may cause phospholipolysis in cell membranes and generate the eicosanoid family of compounds which are proinflammatory in nature and activity (Balsinde *et al*., 1999). The presence of macrophages at the arthropathic foci also strengthens the above cells destruction through electrolytes mediation. If calcium were to play crucially the destructive histolysis in arthropathy besides other cell mediated and humoral factors it is conceivable to prevent the focal degeneration through calcium channel blocking mechanism. Towards this, studies have documented the stress induced electrolytes imbalance in several animal models (Ramalingam *et al*., 1980; Anbarasu and Ramalingam, 1992; Rafe *et al*., 1994). The alteration in trace elements, during arthritogenesis is thus a diagnostic pointer to the focal homeostasis disturbances, like musculo-skeletal region.

Therapeutic considerations

Since arthritic process involves both cellular elements and humoral components such as IL-1 and TNF (cytokines) it is suggested that combination therapy may be advisable and statutory. Several experimental evidence revealed the basic approaches to the alleviation of arthritis.

1) In one animal model the synovial lining macrophages from the murine knee joint have been selectively eliminated by local application of phagocytosable toxic liposomes before arthritis induction. This treatment has almost completely reduced the onset of arthritis in the joint but did not fully control the destructive process. (Gaston, 1999). Synovial lung macrophages comprise different kinds of subsets with more destructive or protective character. The delineation of such cells with regard to CD markers, may promise novel therapeutic targets.
2) In another line of thinking use of protease inhibitors is suggested to control cytokine-independent enzyme activities in the synovial tissue.

Monoclonal antibody therapy

The role of cytokines in inflammatory arthritis has been unequivocally proved. The principal pro-inflammatory cytokines include IL-1, TNFα, IL-12 and IL-15. In contrast the regulatory cytokines include IL-10 and transforming growth factor beta (TGF β). Considering the above, in vivo predicament, in regard to regulatory versus proinflammatory cytokines, the phase of arthritis development may well be controlled by monoclonal antibody therapy at the initial stage itself. Several experimental tests have been made in these lines viz., anti IL-1 therapy and Anti TNF therapy using monoclonal antibodies (Elliott *et al*., 1993; May *et al*., 1993; Paleolog *et al*., 1996; Vande loo *et al*., 1992b, Kavanaugh *et al*., 1994).

IL-15, an IL-2 homologue produced by T-cells is abundantly present in RA synovia. IL-15 triggers the production of TNF by the macrophages. The induction of macrophage TNF production by IL-15 activated T cells appeared to be dependent on cell-cell contact and was linked to CD 69 expression. CD 69 is also pivotal in the cell-cell interaction of T cells and synoviocytes resulting in cartilage-degrading protease release (Lacraz *et al*., 1994). This new pathway of T-cell derived TNF production sheds new light on the critical T cell involvement in the rheumatoid arthritic lesion process. Hence anti-IL-15 antibodies in RA patients and animal models may reveal both the etiological role of this cytokine and also its ameliorative effect. Already the clinical trials in animal models have revealed the feasibility of monoclonal antibodies in the therapy of rheumatoid disease. Maini et al., (1999) have studied the effect of Influximab (a chronic anti-tumour necrosis factor α mab) in RA patients receiving concomitant methotrexate.

Therapeutic drugs and treatment

The main scopes of therapeutic treatments for joint diseases of any category for that matter include i) relieving the chronic/acute pain, ii) reduction of inflammation, iii) preservation of the functional status of the musculo-skeletal joints and their foci; iv) prevention of complications of the disease, v) resolution of the etiopathogenic process, vi) facilitation of healing and vii) avoidance of complications related to therapy (Robert, 2000). The pharmacologic therapy involves medications such as non-steroidal anti-inflammatory drugs (NSAIDS), glucocorticoids, disease modifying antirheumatic drugs (DMARDS) immunosuppressive drugs and biological agents/molecules such as mAbs. Towards the above much remains to be done with regard to the therapeutic aspects of mAbs.

The non-pharmacological interventions/therapy include a variety of physiotherapeutic techniques aimed at joint protection, optimization of biomechanics, range of motion maintenance, muscle strengthening, joint replacement surgery and other surgical interventions such as synovectomy, arthrodesis, and correction of soft tissue abnormalities (Ayers and Short 1993; Bogoch, 1991). Other modalities/methodologies of treatment include selective inhibition of key proteases; blockade of adhesion molecules/homing receptors or T cells; blockade of T cell effector functions viz., inhibition of T cell produced cytokines and inhibition of Fas-FasL interactions; deletion and/or inactivation of autoaggressive T cells through systemic or mucosal administration of protein or peptide which can induce tolerance to the same proteins. Nutrionwise, the intake of micronutrients, Vitamins D, C and Selenium, Polyunsaturated fatty acids especially n-3 fatty acids have been suggested to ameliorate arthritis/arthropathy. Recently glucosamine and chondroitin have been included in drug schedule to arthritis.

Limitations of idiotypic antibodies

The idiotypic antiauto antibodies may have their own limitations in the in vivo environment. These include,

a. They may sometimes complx with autoantibodies plus complement and may be providing antigenic stimulus to regional lymphnodes to make things still more complicated.

b. Monoclonal antibodies prepared against the cell receptors may have differential binding specificity i.e., binding to immuno-dominant epitopes alone or to other epitopes.
c. The prospects of heterogenous idiotypic monoclonal antibodies killing specific β-cell populations involved in the autoimmune expression are very remote.

However the above adverse features to arthritis need experimental evidences as has been gathered for experimental autoimmune Myasthemia gravis (EAMG) (Engel, 1984; Drachman et al., 1987; Vincent 1987; Vincent et al., 1987)

Conclusion

The musculo skeletal diseases have a strong basis of auto-immune mechanism involving organ/region specific autoantibodies (immunoglobulins). Besides, inappropriate activation of T cells is an involved mechanism in autoimmune diseases particularly the rheumatoid arthritis. Hence mAbs synthesized against CD_4 antigens of T cells may suppress the above T cell functions. In animal models the above therapeutic strategies have proved effective. However similar strategies are yet to be worked out in humans. To this end, monoclonal antibodies against various proteinaceous factors such as monokines, lymphokines, TNF etc will be the future therapeutic strategy and major new development alongside T cell silencing. Considering the dimensions and profiles of arthritic disease manifestations and the susceptible human population at all age groups, monoclonal antibodies may represent the preferential and preemptive approach towards alleviation of these diseases in its own right.

How far the neutralizing therapeutic monoclonal antibodies produced against T cell epitopes or the interleukins/cytokines are efficacious without involving toxicity reactions in vivo need elaborate animal model studies and clinical trials in human subjects. Generally monoclonal antibodies are considered less toxic than other modes of treatments especially the chemotherapy. The mechanism independent toxicities include hypersensitive/anaphylactic reactions. On the contrary, mechanism dependent toxicities may involve organs other than the focused regions. Such reactions have been elucidated in cancer treatment (Byrd et al., 1999; Willet et al., 2004). Despite such bottle necks, it is to be hoped that the modern devices of mAbs technology and engineering could overcome the obstacles in the therapeutic highway research.

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