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Human Cartilage gp-39 as Candidate Autoantigen for Immunotherapy of Rheumatoid Arthritis

Introduction

We have recently described a novel arthritis model in the Balb/c mouse with mild and chronic features (Verheijden et al., 1997). Arthritis was induced by injection of a cartilage-derived antigen, Human Cartilage glycoprotein-39 (HC gp-39), mixed in Incomplete Freunds Adjuvant (IFA). A single injection of protein in female Balb/c mice, induced clinical signs of arthritis first observed in forepaws and a few days later in hind paws. Arthritis activity was followed for 250 days and was characterized by regular peaks of clinical disease. The incidence of HC gp-39-induced disease was 100%. Very often a symmetrical distribution of afflicted joints was observed. Thus, HC gp-39 mixed in adjuvant can trigger a chronic, pathological response resembling RA. Moreover, inhalation of HC gp-39 led to tolerization of antigen-specific T-cells, as assessed in a DTH assay, and more importantly to suppression of HC gp-39-induced arthritis. The data suggest that HC gp-39 may be a relevant autoantigen for immunotherapy of RA. For efficient downmodulation of the autoantigen-specific, pathogenic response in genetically susceptible humans, the identification of T-cell epitopes is required. Preferably, T-cell epitopes to be used for tolerization should be disease-related, MHC class binding (with high affinity for the RA-associated DR molecules), dominantly selected, and naturally processed by APC.

HC gp-39 autoantigen expression in RA

Regardless of what may trigger the autoimmune process in RA, clinical evidence supports the notion that chronic inflammation is sustained by the mere presence of articular cartilage (Laskin, 1990, Panayi, 1993). Proteins differentially expressed at the site of chronic inflammation may, therefore, be involved in RA pathogenesis. HC gp-39 mRNA is detected in synovial or cartilage specimens from RA patients but not in normal articular cartilage obtained at surgery (Hakala et al., 1993). We reasoned that altered expression of HC gp-39 would be associated with the outgrowth of specific T cells in RA patients and might be responsible for maintaining chronic

inflammation. Thus, epitopes of HC gp-39 could be involved in sustaining the chronic inflammatory response.

DR4 (B1*0401)-restricted T-cell epitopes of HC gp-39

The strategy followed for identification of relevant autoepitopes within HC gp-39 was based on the assumption that the DR4 or DR1 molecules predispose to RA (Gao et al., 1990) at two levels. Firstly, by shaping the T cell repertoire and secondly, by determinant selection. The shared epitope found among the RA-associated DR molecules might be involved in selection of similar sets of peptides for presentation to T cells (Gregerson et al., 1987). Putative binding sequences within the primary structure of HC gp-39 were identified by use of a DR4 (DRB1*0401) peptide binding motif (Verheijden et al, 1997). HC gp-39, a protein of 362 aa, excluding the signal sequence (Niyrkos & Golds, 1990), contains six regions accommodating this motif. Since most MHC class II-restricted T-cell epitopes comprise 9-13 aa, we chose to synthesize peptides with a minimal length of 13 aa. Four peptides thus selected were synthesized and tested for binding the RA-associated DR1 and DR4 (DRB1*0401) variants. All motif-based peptides, spanning residues 103-116, 259-271, 263-275 and 326-338 of HC gp-39, were found to bind with high relative affinity to DR4 (DRB1*0401) molecules. The same peptides bound with good or high (263-275) relative affinity to DR1 (DRB*0101).

Subsequently, we examined recognition of these peptides by peripheral blood T cells from RA patients and healthy donors (all positive for the RA-associated DR molecules). Three motif-based peptides, 103-116, 263-275 and 326-338, were preferentially recognized by PBMC from RA patients, thereby suggesting a higher frequency of HC gp-39-specific T cells in RA. To characterize the T-cell response to HC gp-39 at the level of MHC restriction, a T-cell clone (H243) was generated following stimulation of PBMC with the 263-275 peptide. Recognition of the 263-275 peptide by this clone was found to be DR4 (B1*0401)-restricted, thereby underscoring the value of the motif used (Verheijden et al., 1997).

Epitope selection and relative dominance in DRB1*0401 transgenic mice

The identification of T-cell epitopes within HC gp-39 was aided by the use of a DR4 (DRB1*0401) peptide binding motif. To test whether these epitopes are naturally selected *in vivo*, we investigated the frequency of recognition of HC gp-39 epitopes in DRB1*0401 (RA-associated DR molecule) transgenic mice (Fugger et al., 1994; Cope et al., 1998). The data show that upon immunization of 0401 transgenic mice with HC gp-39, T-cell hybridomas generated from popliteal lymph node cells were found to respond dominantly, but not exclusively, to the 3 motif-selected, HC gp-39 epitopes (103-116, 263-275 and 326-338) previously defined. These observations are fully in agreement with the notion that the motif-selected epitopes can be naturally selected, processed and presented by APC *in vivo*. Further analysis of

the HC gp-39-specific response at the cytokine level in these mice was indicative of a Th1-type of response.

Is DRB1*0401-restricted recognition of HC gp-39 associated with an inflammatory type of response in man?

Antigen-specific stimulation of clone H243 reactive to HC gp-39 (263-275) resulted in secretion of high amounts of IFNγ as opposed to IL-4 (Verheijden et al., 1997). The cytokine production profile of the H243 clone is thus characteristic of a Th1-like function. We next investigated whether the polyclonal response to HC gp-39 was associated with an inflammatory type of response in DRB1*0401-positive individuals. For this purpose an Elispot assay for detection of antigen-specific IFNγ and IL-4 production by PBMC from DRB1*0401-positive individuals was employed. Our preliminary data show that stimulation of PBMC with HC gp-39 favours a Th1 type of response which could be of relevance to RA pathogenesis (Miltenburg et al., 1992; Quayle et al, 1993; Simon et al, 1994).

In conclusion, our data indicate that there is a higher frequency of HC gp-39 reactive T cells in DRB1*0401-positive RA patients when compared to DRB1*0401-matched healthy controls. In addition, preliminary data show that the response to HC gp-39 in DRB1*0401-positive individuals is associated with the production of proinflammatory mediators, thereby suggesting that HC gp-39 recognition may be involved in perpetuation of the chronic inflammatory process as seen in RA. Consequently, HC gp-39 is a prime candidate for antigen-specific immunotherapy of RA.

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