14-028

MEMBRANE EXPRESSION OF PROTEINASE 3 IS GENETICALLY CONTROLLED

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Proteinase 3 (PR3) is present on the surface plasma membrane of a subset of PMN. A large PR3-positive subset favors the development of ANCA vasculitis. Earlier studies suggested that PR3 membrane expression is influenced by genetic variance. We tested the hypothesis that membrane PR3 expression on PMN is genetically influenced. We recruited 11 pairs of identical twins (mz; 4m 18f, 31 ± 10 years old) and 7 pairs of fraternal twins (dz; 5m 9f, 26 ± 6 years old). PMN were isolated and PR3 membrane expression was assessed by FACS using a mab to PR3 (CLB-7). In addition, PR3-positive and PR3-negative cells were separated by cell sorting and the intracellular PR3 content was estimated by Western blot analysis. Repetitively FACS analysis performed in 15 healthy subjects showed an excellent correlation in 2 independent investigations (r = 0.91). Assaying the percentage of PR3-positive PMN, we found a highly significant correlation of r=0.991 within mz twins. In contrast, no correlation at all was detected within dz twins (r=0.05). Furthermore, we studied whether or not the difference in membrane expression was the consequence of a different intracellular PR3 content. Western blotting showed similar intracellular PR3 amounts in membrane PR3-positive and membrane PR3-negative cells separated by FACSsort. In summary, our data from a small twin analysis clearly demonstrate that the membrane expression of PR3 is genetically influenced. Furthermore, our data suggest that PR3 membrane expression is not a function of the intracellular PR3 content.

15-015

GENETIC POLYMORPHISMS IN TNF, IL-1, IL-6 AND CYTOTOXIC T LYMPHOCYTE-ASSOCIATED ANTIGEN 4 (CTLA-4) IN WEGENER'S GRANULOMATOSIS (WG)

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Background: Although the precipitating event(s) that trigger WG are unknown, evidence for genetic predisposition has been suggested by the occurrence, albeit infrequent, of familial cases. Cytokines and co-stimulators control the quality and intensity of immune responses. Thus they are relevant candidates for the study of immune dysregulation in WG that may have either an acquired or inherited basis.

Patients and Methods: Using PCR-based, genomic DNA genotyping, this study investigated the polymorphisms located in the genes encoding proinflammatory cytokines such as TNF-α, IL-1 and IL-6, and CTLA-4 (a negative co-stimulator for T cell activation), in 117 American patients with WG and 123 ethnically matched healthy controls.

Results: A significantly lower percentage of patients homozygous for the shortest allele 86 in the microsatellite polymorphism (AT)n located in the 3'-untranslated region of exon 3 of CTLA-4 was found as compared with healthy controls (47% versus 70%, p = 0.0005). Examination of a bi-allelic exonal polymorphism in CTLA-4 did not show skewing in patients. Significant differences in the allelic and genotypic frequencies of polymorphisms in the other proinflammatory cytokine genes studied were not found between patients and controls.

Conclusion: The CTLA-4 (AT)n 86 allele has been previously demonstrated to be crucial for maintenance of normal levels of CTLA-4 expression and T cell activation. Our results confirm findings from a Scandinavian cohort, suggesting that this observation is not limited to WG patients who are ethnically unique. The (AT)n polymorphisms in the CTLA-4 gene may represent a WG-related susceptibility mutation and account for increased T cell activation and clonal expansion in WG patients. Blockade of T cell costimulation using CTLA-4Ig might be a useful therapeutic intervention, providing an alternative or complementary approach to conventional immunosuppressive agents.