8-052

CHEMOKINE RECEPTOR EXPRESSION ON CD4+ AND CD8+ MEMORY T-CELLS AND IN GRANULOMAS IN WEGENER'S GRANULOMATOSIS

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Objective: Upregulated expression of receptors for inflammatory chemokines enables T cells to enter sites of inflammation. We analyzed whether peripheral blood and tissue T cells express chemokine receptors suggestive of their capability to respond to chemotactic gradients and of coordinated T-cell migration in Wegener's granulomatosis (WG).

Methods: Patients with biopsy-proven localized WG (n=5), generalized WG (n=16) and age- and sex-matched healthy controls (HC, n=13) were analyzed. PBMC were isolated and labeled with fluorochrome-conjugated monoclonal antibodies for cell surface antigens or appropriate negative (isotype) controls. Expression of chemokine receptors CCR3, CCR5 and CXCR3 was determined by four-color flow cytometric analysis (FACS). Lymphocytes were gated for analysis based on light-scattering properties and on CD45, CD4 and CD8 staining. Positively and negatively stained populations were calculated by quadrant dot plot analysis determined by isotype controls. CD3 and CCR5 staining of granulomas was done using immunohistochemistry.

Results: The fractions of CCR5+ and CCR3+ cells within the CD4+CD45RO+ and CD8+CD45RO+ T-cell population were significantly expanded in localized and generalized WG as compared to healthy controls. The ratio of CCR5/CCR3 expression on CD4+ and CD8+ memory T-cells and on CD28- T-cells was higher in localized WG compared to generalized WG. CCR5 was also expressed in granulomas on T-cells.

T-cell subset/ mean %	Localized WG	Generalized WG	HC
CD4+CD45RO+CCR5+	15%	4%	1.5%
CD8+CD45RO+CCR3+	50%	5%	2.8%
CD4+CD45RO+CCR5+	7%	5%	1.2%
CD8+CD45RO+CCR3+	10%	4%	1.4%

Conclusion: Upregulated CCR5 and CCR3 expression on memory T cells indicates activation and homing capability of CD4+ and CD8+ memory T cells in WG. Together with CCR5 expression on T cells in granulomas, these findings suggest that expanded Th1 and Th2 effector memory T cell populations home to the pathogenic site with apparent differences between localized and generalized WG.

9-016

GENETIC RESISTANCE TO WEGENER'S GRANULOMATOSIS—A ROLE FOR CCR5 IN PATIENTS WITHOUT ANTINEUTROPHIL CYTOPLASMIC ANTIBODIES

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Background: During inflammation, chemokines control emigration of leukocytes via their G-protein-coupled cell surface receptors. CC chemokine receptor 5 (CCR5) is the receptor for the β-chemokines including RANTES, MIP-1α and MIP-1β. CCR5 is expressed mainly on macrophages, Th1 T cells and dendritic cells. CCR5 Δ32, a naturally occurring variant of CCR5, has a 32 bp deletion (Δ32) that results in a nonfunctional receptor. Leukocytes from individuals heterozygous for CCR5 Δ32 express significantly lower levels of CCR5.

Objective: To investigate whether the expression of CCR5 and its ligands is altered in affected tissues and whether genetic variations in genes for CCR5 and its ligands confer susceptibility to WG.

Patients: One hundred eighteen Caucasian patients with WG and 127 ethnically matched healthy controls were included in the genetic analysis. Four lung biopsies that had classical features of WG were examined for protein levels of CCR5 and its ligands.

Measurement: Genomic DNA samples were amplified using PCR-based method. CCR5 Δ32, RANTES –28 and –401 polymorphisms were determined by either specific primers or direct sequencing. Tissue protein levels of CCR5, RANTES, MIP-1α and MIP-1β were examined using immunohistochemistry.

Results: $CCR5^+$ cells were enriched in lung lesions from patients with WG. Among patients in whom circulating antineutrophil cytoplasmic antibodies (ANCA) were repeatedly absent, none were found to carry the CCR5 Δ32 allele. The significant under-presentation of CCR5 Δ32 in patients without ANCA (0/25, 21.9% of WG cohort) suggests that CCR5 signaling exerts an important and perhaps critical role, with maximal impact in WG patients in whom the influence of ANCA is minimal. Among all patients, patient subsets and controls, there was no significant difference in the frequency of polymorphisms located in the promoter regions of the gene encoding RANTES. Enhanced protein levels of three CCR5 ligands RANTES, MIP-1α and MIP-1β were all noted in WG lung lesions, indicating redundancy of ligands for CCR5 in affected tissue. Taken together, these results demonstrate a critical role for CCR5 in tissue inflammation and destruction in WG.