(Anti-)TNF alpha matters in rheumatoid arthritis

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COLOR FIGURES

CHAPTER 3

Figure 1. A) Visualization of expression of 189 genes with at least a 1.4 fold difference in expression between responders and non-responders (indicated at the top). Red indicates a high level of expression, green a low expression level, black indicates intermediate expression and grey indicates missing data points. The red bar at the right indicates a cluster of correlated genes ($R=0.79$) with a higher expression in responding patients' samples.

B) Enlarged view of genes with a high correlation across the patient samples ($R=0.79$), indicating increased expression of inflammation-related genes in the responder group.
Figure 2. Synovial Apoptosis. A and B, Number of TUNEL+ cells/mm² in paired synovial tissue sections obtained from 5 patients with rheumatoid arthritis (pat1–pat5) before treatment (T = 0) and 1 hour after infliximab treatment (A) and from 5 patients (pat6–pat10) before treatment and 24 hours after treatment (B). There was a minor increase in the number of TUNEL+ cells/mm² in 2 patients and a decrease in 3 patients 1 hour after treatment. There was an increase in the number of TUNEL+ cells in 3 patients 24 hours after treatment. In 1 patient only (patient 9) this was associated with an increase in the number of active caspase 3+ cells and the number of apoptotic cells, determined by electron microscopy. None of the differences were statistically significant. C–F, Staining for TUNEL cells (purple staining) in paired synovial tissue sections obtained from 2 patients before treatment (C and E) and 24 hours after treatment (D and F). No increase in the number of TUNEL+ cells was seen in 2 of 5 patients (C and D). A slight increase in the number of TUNEL+ cells was seen in 3 of 5 patients 24 hours after the first infusion (E and F).
Figure 2. Schematic view of the interaction between MIF and TNF.

Both macrophages and T cells as well as dendritic cells and fibroblast-like synoviocytes produce MIF and TNF. TNF induces the production of MIF, and vice versa TNF production can be induced by MIF. [8, 17] In RA increased levels of MIF and TNF have been found locally in the synovial fluid and synovial tissue, which perpetuate the inflammatory process not only by inducing further cytokine secretion, but also by enhancing leukocyte migration towards the site of inflammation. [19] With anti-TNF antibody therapy available bioactive TNF is neutralized. Furthermore, the infiltration of the inflamed synovium by macrophages (main producers of TNF and MIF) was shown to diminish early after treatment. [27] Hence, both the number of MIF producing cells as well as the concentration of bioactive TNF decreases after anti-TNF therapy potentially leading to a decrease in systemic MIF levels.
Figure 1. (A) Cluster diagram of the expression of 577 significantly different expressed genes in 35 patients and 15 healthy individuals. Genes are organized by hierarchical clustering based on overall similarity in expression patterns. [18] Red represents relative expression greater than the median expression level across all samples, and green represents an expression level lower than the median. Black indicates intermediate expression. Grey indicates missing data. Colored bars to the right identify the locations of a category of clustered genes, with a correlated expression profile and related function. (B) Representation of the IFN-response gene cluster with an enhanced expression in the group having patients with rheumatoid arthritis (RA). An expanded view of the genes in the IFN-response cluster of (A) is shown. Genes are either known genes with a unigene symbol characteristic for the defined gene cluster, or genes are unknown, indicated by an accession number or unigene cluster ID. (C, D) The IFN-response program is present in patients with RA irrespective of treatment. Representation of genes that are expressed at significantly different levels between patients with RA undergoing (C) or patients not undergoing (D) methotrexate (MTX) treatment and age-and sex-matched healthy controls. A selection of genes with a correlated expression profile that are indicative for an IFN-response program is shown.
Figure 3. A subgroup of patients with rheumatoid arthritis (RA) show increased expression of IFN-response genes (IFN$^{\text{high}}$). Each square represents a single individual with the average expression ratio of all 43 IFN-response genes, which are shown as a distinct cluster in fig 1A and B. The shaded box indicates the normal range within the 95% confidence limits. Patients with RA outside the shaded box are defined as the IFN$^{\text{high}}$ group. HC, healthy control.
CHAPTER 10

Figure 1. Different patterns of lymphocyte infiltration can be detected in the synovial tissue. In a proportion of patients a mixed infiltration of aggregates of T and B cells is present (A and B), together with a high number of infiltrating macrophages (C). Both TNFα (D) and LTβ (E) are abundantly expressed. In other patients a diffuse or scarce infiltrate of CD3+ T cells is found (F), and little or no B cells (G), while macrophages are the dominant infiltrating cell population (H). TNFα and LTβ are expressed at low levels in these patients (I, J). (Original magnification × 20.)

Figure 2. In 8% of the synovial tissue samples, clusters of follicular denritic cells, expressing the CD21 long isoform (A), could be detected in lymphocyte aggregates enriched with CD22 positive B cells (B). (Original magnification × 20, inset × 40.)
CHAPTER 13

Figure 1. MIF expression in synovial tissue A) 3 examples of immunofluorescent staining of MIF (ALEXA-red) in the endothelium. B) CD68+ macrophages (FITC-green), MIF (ALEXA-red), and double staining of CD68 and MIF. C) CD3+ T cells (FITC-green), MIF (ALEXA-red), and double staining of CD3 and MIF. DAPI was used to stained the nucleus blue (unpublished Figure).