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Souhrn

Cíle práce: Cílem této práce bylo zhodnotit parametry buněčné imunity u pacientů s ANCA (Anti-Neutrophil Cytoplasmic Autoantibodies – autoprotilátky proti cytoplazmě neutrofilů)-asociovanými vaskulitidami (AAV) v různých stádiích onemocnění, s různou terapií a s ohledem na jejich dlouhodobou prognózu.

Metodika: Vyšetřeno bylo 69 pacientů s AAV, 30 zdravých jedinců a 20 pacientů s chronickým onemocněním ledvin. Metodou průtokové cytometrie jsme u buněk periferní krve stanovovali následující markery: povrchové molekuly (CD4, CD8, CD3, CD19, CD80, CD86, HLA-DR, CD28, CXCR3, CCR5, CD30 a CRTH2) a intracelulární cytokiny (interferon gama (IFNγ), tumor nekrotizující faktor alfa (TNFα), interleukin (IL)-2 a IL-4 v CD3+ T buňkách a IL-10 a IL-12 v monocytech).

Výsledky: Pacienti s AAV měli ve srovnání se zdravými kontrolami snížený celkový počet lymfocytů, CD4+ buněk a CD4+CD45RA+ buněk ($p<0.001$). Aktivní pacienti měli zvýšenou expresi molekul CD30 a CRTH2 ($p<0.05$). Zvýšená exprese CCR5 přetrvávala i v remisi. Zvýšená exprese HLA-DR, expanze CD28– subpopulace a zvýšená produkce IFNγ byly pozorovány v remisi, ale ne u aktivní AAV. Pacienti v remisi, u kterých byl během dlouhodobého sledování zaznamenán relaps, měli významně nižší produkci IL-10 než ti bez relapsu ($p<0.01$).

Závěry: Naše data celkově prokazují perzistující aktivitu imunitního systému v remisi AAV, což svědčí o důležitosti udržovací imunosupresivní terapie v remisi. Nízká produkce IL-10 v remisi je v dlouhodobém sledování asociována s vyšším výskytem relapsu.

Summary

Objectives: The aim of this study was to assess cellular immunity parameters in patients with ANCA (Anti-Neutrophil Cytoplasmic Autoantibodies)-associated vasculitides (AAV) at different stages of the disease, with different treatment modalities, and with respect to the long-term prognosis of the patients.

Methods: We examined 69 patients with AAV, 30 healthy individuals and 20 patients with chronic kidney disease. Using flow cytometry, the following markers were assessed in peripheral blood cells: surface molecules (CD4, CD8, CD3, CD19, CD80, CD86, HLA-DR, CD28, CXCR3, CCR5, CD30 and CRTH2) and intracellular cytokines (interferon gamma (IFNγ), tumor necrosis factor alpha (TNFα), interleukin (IL)-2 and IL-4 in CD3+ T cells and IL-10 and IL-12 in monocytes).

Results: Patients with AAV had decreased total number of lymphocytes, CD4+ cells, and CD4+CD45RA+ cells compared to healthy controls ($p<0.001$). Active patients had increased CD30 and CRTH2 expression ($p<0.05$). Increased CCR5 expression persisted in remission. Increased HLA-DR expression, expansion of CD28– subpopulation and increased IFNγ production were noted in remission but not in active disease. Patients in remission who developed a relapse during follow-up had significantly lower IL-10 production than those without relapse ($p<0.01$).

Conclusions: Taken together, our data demonstrate the persistent immune system activation in remission of AAV and indicate the importance of ongoing immunosuppressive treatment in remission. Low IL-10 production in remission is associated with a higher relapse rate in the long-term follow-up.

1. Introduction

Vasculitides are a heterogeneous group of clinico-pathologic units characterised by inflammatory infiltration and potential destruction of blood vessel walls. Wegener's granulomatosis (WG), microscopic polyangiitis (MPA) and Churg Strauss syndrome (CSS) are immune-mediated diseases with a strong and highly specific association with Anti-Neutrophil Cytoplasmic Autoantibodies (ANCA). Together with idiopathic (isolated) rapidly progressive glomerulonephritis (iRPGN) they are therefore all ranked among ANCA-associated vasculitides (AAV).

ANCA are predominantly IgG autoantibodies directed against different target antigens located in azurophilic granules of polymorphonuclear leucocytes and the peroxidase-positive lysosomes of monocytes. In WG, ANCA are usually directed against proteinase 3 (PR3-ANCA) and have a cytoplasmic type of immunofluorescence (c-ANCA). In MPA the target antigen is mostly myeloperoxidase (MPO-ANCA) and the type of immunofluorescence is perinuclear (p-ANCA). Both MPO-ANCA and, less frequently, PR3-ANCA can be also found in patients with CSS and iRPGN.

The annual incidence of AAV in Europe is approximately 10-20/million. AAV typically involve kidney, lungs and upper respiratory tract even though, in principle, any organ may be afflicted. Diagnostics is based on clinical picture,

ANCA testing and—when possible—verification by a biopsy. Untreated, generalised WG and MPA follow a progressive course with a fatal outcome due to vital organ failure. Standard combined immunosuppressive treatment consisting of cyclophosphamide and corticosteroids leads to successful remission achievement in most patients. Side effects of the therapy are, however, common; and, in the long run, many of the patients relapse.

The pathogenesis of ANCA-associated vasculitides remains to be elucidated. Nevertheless, it seems to be a complex process which likely involves impaired immune mechanisms of both innate and adaptive, and both humoral and cellular immunity.

2. Objectives

Whilst most authors focused on the role of ANCA in the pathogenesis of AAV, the cellular immunity in AAV has only recently attracted attention. This flow cytometric study therefore aimed to assess various cellular immunity parameters (i.e. lymphocyte subpopulations, surface molecule expression, and intracellular cytokine production) in patients with AAV at different stages of the disease, with different treatment modalities, and with respect to the long-term prognosis of the patients.

3. Material and Methods

A total of 69 patients with AAV were originally included into this study during a 16-month period (September 2004 – December 2005). The basic characteristics of the patients are summarized in Table 3.1. The patients' initial status was noted and all patients were subsequently monitored up until May 2008 at their regular visits to the clinics.

Table 3.1: Patient characteristics at the time of inclusion into the study (No. of patients = 69).

Characteristics		**Data**[1]
Age (years)		56 (22-76)
Male gender		40 (58%)
Type of vasculitis		
	WG	39 (56.5%)
	MPA	19 (27.5%)
	RLV	9 (13%)
	CSS	2 (3%)
ANCA specificity		
	PR3-ANCA	42 (60.9%)
	MPO-ANCA	27 (39.1%)
Duration of vasculitis before inclusion (months)		15 (0-168)
Organ involvement (anytime in patients' history)		
	Kidney	68 (98.5%)
	Lungs	36 (52.2%)
	ENT	25 (36.2%)
Serum creatinine (μmol/L)		173 (46-602)
No. of patients on haemodialysis		14 (20.3%)

[1]Data are presented as No.(%), or median (range). ENT = ear, nose, throat. For other abbreviations see text.

Blood samples were collected from each patient at entry. Some of the patients were examined repeatedly when their disease activity changed.

Thus, a total of 94 analyses of peripheral blood samples of 69 patients with AAV were performed. A total of 43 patients were examined in the active phase of the disease (20 patients with newly diagnosed disease, 9 patients with chronic grumbling disease and 14 patients with relapse). Fifty-one patients were examined in remission. In 26 patients (10 with active disease) no immunosuppressive treatment was administered at the time of examination. During the long-term follow-up, relapse was observed in 19 patients out of 51 patients originally examined in remission.

The control groups consisted of 30 healthy volunteers or blood donors and of 20 patients with chronic kidney disease.

The following monoclonal antibodies were used for flow cytometric analysis of cell surface molecules: CD4-PerCP (peridinin chlorophyll protein), CCR5-PE (phycoerythrin), CD19-PerCP, CD80-PE, CD86-PE, CD3-FITC (fluorescein isothiocyanate), HLA-DR-PE, CD45RA-FITC, CD28-PE, CD30-PE, (all commercially available from BD Biosciences, San Jose, CA, USA), CRTH2 (Th2 selective chemoattractant receptor-homologous molecule expressed on Th2 cells)-PE (Immunotech-Beckman Coulter, Fullerton, CA, USA) and CXCR3 (CXC chemokine receptor 3)-FITC (R&D, Minneapolis, MN, USA).

The production of intracellular cytokines after cell stimulation was also assessed by flow cytometry. For the sample staining, the following monoclonal antibodies (BD Biosciences) were used: CD3-PerCP, TNFα (tumor necrosis factor alpha)-FITC, IL (interleukin)-2-PE, IFNγ (interferon gamma)-FITC, IL-4-PE, IL-10-PE, IL-12-PE and CD14-FITC. All samples were analyzed by FACSCalibur flow cytometer using three-colour imaging. T lymphocytes were gated using FSC (forward scatter) and CD3 whereas monocytes were gated using SSC (side scatter) and CD14.

Statistical analysis was performed applying SPSS and STATGRAPHICS Plus software. Differences in continuous variables were compared by means of Student's *t*-test in the case of normal distribution. Otherwise, the Wilcoxon signed rank test or the Mann-Whitney test were used. For multiple comparisons, the non-parametric Kruskal-Wallis test or ANOVA were applied. The correlation analysis was performed using the non-parametric Spearman's test. A two-sided $P < 0.05$ was considered statistically significant in all cases.

4. Results

Results of cell surface molecule expression and intracellular cytokine production in AAV patients and healthy controls are compared in Table 4.1.

No correlation between age and laboratory parameters was observed in the correlation analysis, nor did glomerular filtration rate significantly correlate with any of the surface markers or intracellular cytokines. ANCA levels significantly positively correlated with the percentage of IL-10 and IL-12 producing cells, and with the percentage of CD19 cells. On the other hand, a significant negative correlation between ANCA levels and CD28–CD8+ cells was observed. Furthermore, BVAS (Birmingham Vasculitis Activity Score) but not ANCA levels significantly positively correlated with the numbers of CRTH2+ and CD30+ cells. A positive correlation between the number of IL-10 producing cells and BVAS was also found.

Patients with AAV in remission had significantly higher percentage of CD8+ cells than patients with active disease ($p<0.05$). They also had higher numbers of activated (HLA-DR+) T cells and CXCR3+CD4+ cells than active patients. On the contrary, the CD19+ cell count and also the number of CD4+45RA+ naïve T cells in remission were significantly lower ($p<0.001$, and $p<0.01$ respectively). Patients in remission but not active patients had a significant expansion of CD28– subpopulation of CD8+ cells.

Table 4.1: Surface molecule expression and intracellular cytokine production in patients with AAV and healthy controls.

	AAV (N=69)	**Healthy** (N=30*)	**P value**
CD4+[1]	40.4 ± 13.5	48.2 ± 7.5	**<0.001**
CD8+[1]	29.0 ± 13.7	22.0 ± 6.0	**<0.05**
CD19+[1]	9.6 ± 8.0	10.4 ± 3.2	n.s.
CD3+HLA-DR+[1]	6.9 ± 6.8	4.6 ± 2.6	**<0.01**
CD4+45RA+[1]	19.6 ± 8.9	30.4 ± 11.7	**<0.001**
CD28–CD8+[1]	21.4 ± 15.3	13.3 ± 5.3	**<0.01**
CD80+CD19–[1]	6.7 ± 4.0	4.9 ± 3.1	**<0.05**
CD86+CD19–[1]	9.0 ± 4.2	6.1 ± 3.4	**<0.05**
CD30+[1]	7.4 ± 4.6	4.6 ± 2.8	**<0.05**
CCR5+[2]	23.0 ± 11.6	16.4 ± 8.4	**<0.01**
CXCR3+[2]	39.3 ± 16.6	37.1 ± 10.0	n.s.
CRTH2+[2]	10.6 ± 7.6	6.5 ± 2.2	**<0.05**
IFNγ[3]	34.8 ± 16.5	29.8 ± 9.8	n.s.
IL-4[3]	4.5 ± 4.0	3.6 ± 3.4	n.s.
TNFα[3]	49.0 ± 24.0	45.4 ± 19.4	n.s.
IL-2[3]	34.5 ± 18.9	31.1 ± 11.8	n.s.
IL-10[4]	14.4 ± 10.3	14.3 ± 9.8	n.s.
IL-12[4]	17.9 ± 13.2	10.2 ± 9.8	**<0.05**

Data presented as mean ± standard deviation. P value represents the difference between patients with AAV and healthy controls. n.s. = not significant, *N=24 for intracellular cytokine production, [1]in % of lymphocytes, [2]in % of CD4+ lymphocytes, [3] in % of CD3+ cells, [4]in % of monocytes.

Furthermore, active patients with AAV had significantly lower production of IFNγ than patients in remission ($p<0.01$). On the other hand, the production of IL-10 was significantly higher in active patients than in remission.

In detailed subgroup analysis, the production of IL-10 was significantly higher in newly diagnosed patients than in patients with relapse but did not significantly differ from healthy controls. In addition, newly diagnosed patients had increased IL-12 production when compared to patients with relapse, and, in this case, also to healthy controls.

In comparison with active patients on immunosuppressive therapy, the active untreated patients examined in this study had significantly lower numbers of CD8+ cells ($p<0.05$). They also had significantly lower percentage of CD80+ cells within the CD19+ subpopulation of lymphocytes ($p<0.01$). Similarly, patients in remission on therapy had significantly higher expression of CD80 both on CD19– lymphocytes ($p<0.05$) and within the CD19+ subpopulation ($p<0.001$) than patients in remission in whom the immunosuppression had been stopped before.

Special emphasis in this study was placed on discerning any lymphocyte subpopulations or cytokine patterns potentially associated with the prognosis. When patients in remission who subsequently relapsed during the follow-up (N=19) were compared to those without relapse (N=32), the IL-10 production was significantly lower in the patients with relapse than in those without ($p<0.01$). However, no correlation

between the length of remission and IL-10 production was observed. There was also no correlation between the IL-10 production and the time to relapse.

5. Discussion

In this study, distinct abnormalities in the numbers and phenotype of peripheral blood lymphocytes as well as in the intracellular cytokine production were found in patients with generalized AAV. Despite somewhat different methodology, our findings strengthen and further extend results of a number of earlier studies.

Compatibly with findings of others (Iking-Konert et al.; Marinaki et al., 2005; Schlesier et al.), patients with AAV had significantly lower total number of lymphocytes, diminished proportion of CD4+ cells and enlarged proportion of CD8+ cells when compared to healthy individuals. Notably, lower numbers of lymphocytes and CD4+ cells were observed in all subgroups of AAV patients, including the untreated active ones. Therefore, immunosuppressive therapeutic regimens themselves cannot be blamed for these findings. We presume that the decrease in the total number of circulating lymphocytes might reflect the initial pathogenic vasculitic process with lymphocyte pooling in peripheral tissues.

Interestingly, the significant expansion of CD8+ cells in this study was observed in remission phase of the disease but not in active patients, in particular not in the untreated ones.

Thus, the influence of administered immunosuppressive therapy on the numbers of CD8+ cells seems likely. However, we show that increased CD8 numbers prevail long after cyclophosphamide treatment has been stopped. Therefore, other factors that account for the expansion of CD8+ cell population in AAV are likely to be identified.

In line with this, upregulated expression of co-stimulatory molecules of tumor necrosis factor receptor superfamily on expanded CD8+ T cells but not on CD4+ cells in WG was previously described by Giscombe et al. This expression might augment clonal expansion and differentiation, and sustain longevity of cytotoxic T cell response.

Furthermore, Iking-Konert et al. reported on the presence of activated CD8+CD28+CD11b+ cells during active vasculitis, giving rise to a more persistent phenotype CD8+CD28–CD11b+ found in remission and under immunosuppressive therapy. Activated CD8+ cells may therefore play an important role in the pathogenesis of AAV.

In accordance with these findings, a significant increase in CD28–CD8+ cells was noted in patients in remission in our study as well as in some previous studies (Giscombe et al.; Moosig et al.). Similarly as others (Iking-Konert et al.), we also observed that low immunoregulatory index (CD4:CD8 ratio) in AAV patients correlated with a high percentage of CD28–CD8+ cells.

Nevertheless, in contrast to observations of others (Moosig et al.), CD28–CD8+ cells were lower in active disease

than in remission in our study; they even negatively correlated with ANCA levels. Taken the previously described findings into account, the explanation might be that in active disease a population of CD8+CD28+ cells prevails and the loss of CD28 expression occurs later in the period of active disease and persists in remission. In that case the timing of blood sample collection during active disease would be important; in our study, most of the active patients were examined soon after the disease activity appeared.

Molecules CD80 and CD86 are CD28 ligands. In this study, the expression of neither CD80 nor CD86 on CD19+ (B) cells in AAV differed from healthy controls. Nevertheless, while B cell numbers generally decreased on therapy, the expression of CD80 per B cell increased. This finding would not be noted if only the total numbers of CD80 expressing B cells were considered. Instead of this, the proportion of CD19+ cells expressing CD80 was calculated in this study (as the percentage of CD80+CD19+ cells divided by the percentage of CD19+ cells, multiplied by 100). In our opinion, such a calculation helps to prevent misinterpretation of the results, e.g. in the case of low CD19+ cell numbers under immunosuppression.

Intriguingly, an increased expression of both CD80 and CD86 on CD19– cells was observed in AAV patients in remission when compared to healthy controls. As the unusual up-regulation of CD80 and CD86 on T cells in patients with AAV had been reported before (Moosig et al.), we supposed

that the CD19 negative population represented T cells.

Both the expansion of CD28– cells and the up-regulation of CD28 ligands on T cells seem to go hand in hand with the ongoing inflammation in AAV patients. The loss of CD28 expression on T cells is considered a consistent biological indicator of aging in the human immune system. It has been noted that in immunopathological processes, including AAV, CD28– T cells likely represent prematurely senescent lymphocytes due to persistent immune activation. As for the increased expression of CD80 and CD86 on T cells, a self-co-stimulation of these cells is, theoretically, conceivable. Thus, the increased expression of co-stimulatory molecules on T cells may lead to peripheral blood T cell stimulation and contribute to the persistent T cell activation in AAV.

The hypothesis of persistent immune system activation in remission of AAV has been supported by several results of our study (lower numbers of CD4+CD45RA+, higher numbers of activated HLA-DR+CD3+ cells, and higher IFNγ production were found in remission). However, the stimulus that triggers persistent T cell activation in remission of AAV remains to be identified. It is also questionable whether this finding is a cause, a bystander phenomenon or rather a consequence of the inflammatory process in AAV.

The explanation for relatively low markers of T cells activation found in active disease in this study is not entirely clear but may comprise the usage of HLA-DR, thought a less

appropriate marker of persistent T cell activation than CD25 by others (Marinaki et al., 2006). On the other hand, HLA-DR and CD25 appear on T cells at different stages of activation. HLA-DR appears later than CD25 and persists for a longer period (Giscombe et al.). Thus, the above-mentioned timing of blood sample examination may have contributed to lower numbers of HLA-DR+ cells in active disease in this study. In theory, more intense immunosuppressive therapy administered in active patients might have also influenced the results; the results of untreated patients did, however, not differ from patients on immunosuppression in the parameters of T cell activation.

The possible Th1/Th2 polarization in AAV has been studied thoroughly before (reviewed in Lamprecht et al., 2005; Sanders et al., 2003). In this study, we were not able to detect outright Th1 or Th2 polarization in patients with generalized AAV. Levels of both Th1- (CCR5, IL-12, IFNγ) and Th2-associated markers (CD30, CRTH2) were increased in patients with AAV when compared to healthy individuals. Nevertheless, while both CD30 and CRTH2 together with IL-10 production prevailed in active disease and correlated with disease activity measured with BVAS, the Th1-associated markers were either similarly increased in both active disease and remission (CCR5, IL-12) or significantly higher in remission than in active disease (CXCR3, IFNγ). Thus, we speculate that Th2 type of immune response may play an important role in the pathogenesis of active generalized vasculitis, whereas Th1 type of immune response seems to

predominate later in the pathogenic process and in remission when Th2 cell numbers in peripheral blood tend to normalize.

In agreement with previous reports (Lamprecht et al., 2003), the increased expression of Th1-associated chemokine receptor CCR5 on CD4+ T cells persisted in patients in remission in this study. The increased expression of CCR5 in AAV has been ascribed to the expanded population of effector memory T cells (T_{EMs}). Most of T_{EMs} in WG lack CD28 expression, and display upregulated CCR5, HLA-DR, CD18 (β_2 integrin/adhesion molecule), CD152 (CTLA-4), and CD57 expression (differentiation marker). The majority of authors agree that CD28– T_{EMs} secrete Th1 cytokines (reviewed in Lamprecht et al., 2006). Even though not all of these markers were measured in this study, we presume that our findings in patients in remission (i.e. low numbers of CD4+CD45RA+ cells, increased numbers of HLA-DR+ cells, increased expression of CCR5, and increased IFNγ production) conform to the presence of expanded population of T_{EMs} in AAV.

Regarding the intracellular cytokine production, one of the prominent findings in our study was the increased IL-12 production in patients with active generalized vasculitis that persisted even in remission, even though a positive correlation with ANCA levels was observed. This is in agreement with previous studies (Ludviksson et al.; Marinaki et al., 2005) and might imply a skewed regulation towards Th1 cell differentiation as IL-12 is a major inducer of IFNγ and as such is necessary for differentiation and proliferation of Th1 cells.

Importantly, IL-10 was the only marker in this study related to the long-term prognosis of patients in the prospective follow-up. As mentioned earlier, many of the patients with AAV who successfully achieve remission undergo a relapse. Identifying patients at risk for relapse is one of the lasting challenges in the field of vasculitides. We demonstrate that low IL-10 production at anytime in remission of the disease is associated with a higher probability of subsequent relapse, which in our study occurred in the range of 2 to 38 months after the cytokine measurement. Similar findings were reported in previous studies (Ohlsson et al.; Sanders et al., 2006).

In accordance with the known significance of activation (e.g. infectious) stimuli in the disease pathogenesis of AAV, it seems that patients with low IL-10 are particularly prone to relapse after an intercurrent infection due to the lack of the anti-inflammatory potential of IL-10. Based on the available data, IL-10 might serve as a marker of imminent relapse in patients with AAV, including those who seem to be in stable remission.

Some markers of immune system activation (HLA-DR and CCR5 expression) were equally elevated in patients with chronic kidney disease (CKD) and AAV patients, or were even higher in CKD than in AAV, in particular than in active AAV (IFNγ and TNFα production). However, no shift in CD4:CD8 ratio was found in CKD. A profound difference between CKD and AAV patients was also observed in CD28 expression. Importantly, in CKD patients neither CD80 nor CD86

expression (increased on CD19− lymphocytes in remission of AAV) differed from healthy individuals. Contrary to active AAV patients, patients with CKD did not display increased CD30 and CRTH2 expression.

Taken together, activation of the immune system is present in CKD but differs from that in AAV in several aspects. We conclude that whilst renal insufficiency may to some extent contribute to persistent T cell activation in AAV, it does not seem to be the crucial factor for two following reasons. Firstly, we observed higher parameters of T cell activation in patients in remission than in active disease even though active patients had generally higher levels of serum creatinine than patients in remission. Secondly, the signs of persistent immune system activation in AAV were also described in previous studies in that renal involvement was missing in the majority of patients (Giscombe et al.).

In conclusion, our own findings and previous published reports of others lead us to a current working hypothesis on the pathogenic mechanisms in AAV (Fig. 5.1) that, indeed, involve both humoral and cellular immune mechanisms. Even though much is already known, the proposed model of the disease pathogenesis provides important clues for future research that might help to identify potential therapeutic targets and improve diagnostic possibilities to predict and early detect AAV relapse.

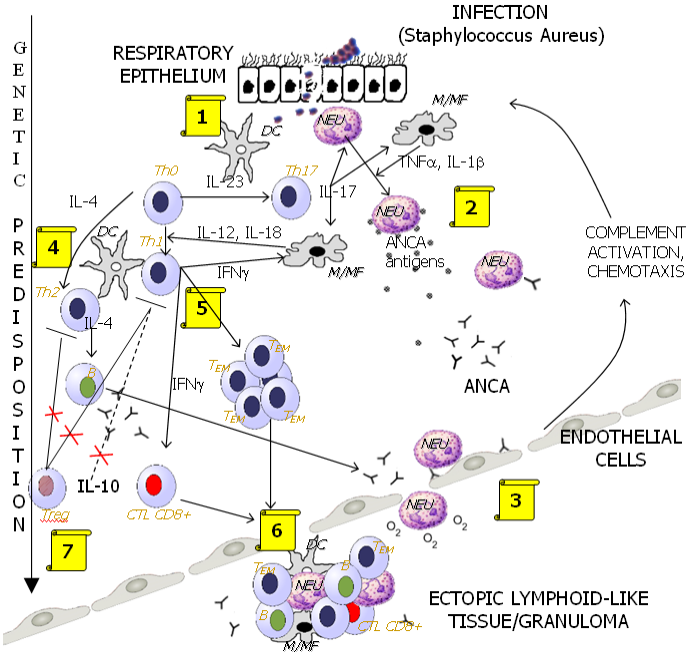

Fig. 5.1: Current working hypothesis on the pathogenic mechanisms in AAV [freely adapted from van Paassen et al.; Lamprecht et al., 2006; Abdulahad et al., 2009].

Neu = neutrophil, DC = dendritic cell, M/MF = monocyte/macrophage, CTL = cytotoxic T lymphocytes. For other abbreviations see text.

(1) The presence of infectious agents stimulates antigen-presenting cells (e.g. DC) to produce IL-23, which induces differentiation and proliferation of Th17 cells. Th17 cells play role in the rapid recruitment of neutrophils and also stimulate monocytes/macrophages to produce pro-inflammatory cytokines. (2) Chemokines and pro-inflammatory cytokines attract neutrophils to the infected tissue and cause neutrophil priming with subsequent release and increased membrane expression of ANCA antigens, which become accessible for interaction with ANCA. (3) The inflammatory cytokines also lead to upregulation of adhesion molecules both on neutrophils and endothelial cells. ANCA activate neutrophils adherent to endothelial cells which results in respiratory burst and neutrophil degranulation. Reactive oxygen species and proteolytic enzymes cause tissue damage. Released antigens also bind to endothelial cells and in the presence of ANCA form transient immune complexes which leads to complement activation and amplification of the immune response. (4, 5) Activated DCs induce both Th2 and Th1 type of immune response. Th2 cells further participate in B cell activation with subsequent ANCA production (4). Exaggerated Th1 type of immune response results in macrophage activation, and plays role in the activation of cytotoxic CD8+ T lymphocytes. Cytokine- and/or antigen-driven expansion of CD28– T effector memory cells (T_{EM}) occurs (5). (6) Both T_{EM} and cytotoxic T lymphocytes directly contribute to the endothelial and tissue damage. Moreover, T_{EM} sustain chronic granulomatous inflammation with ectopic lymphoid-like tissue formation, which further promotes ANCA formation. (7) Genetic predisposition helps to sustain the inflammatory process. Regulatory immune mechanisms with impaired function fail to resolve inflammation in a normal manner.

6. Conclusions

- Patients with AAV, including the untreated ones, had decreased total number of lymphocytes and of CD4+ cells in peripheral blood. This may reflect increased numbers of these cells found in the inflammatory lesions in AAV.
- An expansion of CD28–CD8+ subpopulation that correlated with low CD4:CD8 ratio and negatively correlated with ANCA levels was noted in remission.
- Increased expression of CD80 and CD86 on CD19– cells (T cells) observed in this study likely contributes to the persistent T cell activation in AAV.
- The presence of T cell activation in remission of AAV was supported by the decrease in CD4+CD45RA+ and the increase in CD3+HLA-DR+ supopulations. These results indicate the importance of ongoing immunosuppressive treatment in remission.
- We did not acquire enough evidence to detect outright Th1/Th2 polarization in generalized AAV.
- Low IL-10 production in remission was associated with increased risk of relapse.
- Mechanisms of immune system activation clearly differ between non-AAV renal insufficiency and AAV.

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List of Publications

1. *Publications in extenso related to the topic of PhD Thesis*

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2. *Publications in extenso not related to the topic of PhD Thesis*

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