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IMMUNOLOGICAL STUDIES ON ANCA-ASSOCIATED VASCULITIS WITH SPECIAL REFERENCE TO T CELL ACTIVATION

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ABSTRACT

This thesis focuses on the role of T cells in ANCA-associated vasculitis. We initially investigated activation markers on T cells in patients with Wegener's granulomatosis (WG), microscopic polyangiitis (MPA) and renal limited disease (RLD) and found increased expression indicating continuous activation despite clinical remission. As apoptosis maintains homeostatic control of lymphocyte populations we examined its role in WG, MPA and RLD in comparison with patients with rheumatoid arthritis, lupus erythematosus and Sjögren´s syndrome. We found that patients with ANCA-positive vasculitis have high serum levels of soluble Fas (sFas) that remain elevated even in clinical remission. In our third paper we studied the influence of chemokines on the cell surface expression of thrombospondin-1 (TSP-1) in T cells. The results demonstrate a chemokine-dependent CD26-controlled regulation of T cell polarity and migration through endogenous TSP-1. Based on the results in the third paper we then studied patients with ANCA positive vasculitis with respect to serum levels of chemokines, T cell mRNA expression of chemokine receptors, and possible influence of CD26 on TSP-1 expression. We found altered chemokine levels and evidence for impaired CD26 suppression of chemokine- dependent TSP-1 expression in vasculitis patients in comparison with patients with asthma suggesting that a mechanism for negative regulation of T cell migration is suppressed in these patients. These findings suggest that T cells play an important role in ANCA associated vasculitis and may be useful targets in the development of therapeutic strategies.

LIST OF PUBLICATIONS

I. T cell activation in patients with ANCA-associated vasculitis: inefficient immune suppression by therapy.
Christensson M, Pettersson E, Sundqvist K. G,Christensson B
Clin Nephrol, 2000; 54:435-442.

II. Serum sFAS levels Are Elevated in ANCA-positive Vasculitis Compared with other Autoimmune Diseases.
Christensson M, Pettersson E, Eneslätt, K, Christensson B, Bratt J
Rantapää-Dahlqvist, S. Sundqvist, KG
J Clin Immunology, 2002;22:220-227

III. A CD26-Controlled Cell Surface Cascade for Regulation of T Cell Motility and Chemokine Signals.
Liu Z, **Christensson M**, Forslow A, De Meester I, Sundqvist KG.
J Immunol, 2009;183: 3616-3624

IV. Impaired CD26 suppression of cell surface Thrombospondin-1 expression in T lymphocytes from patients with autoimmune vasculitis.
Christensson M, Bergström SE, Skedinger M, Liu L, Forslöw A,
Pettersson E, Sundqvist KG
Submitted

CONTENTS

LIST OF ABBREVIATIONS

Antineutrophil cytoplasmic antibodies	**ANCA**
Antigen presenting cells	**APCs**
Cytolytic T cells	**CTLs**
Cytotoxic T lymphocyte-associated protein-4	**CTLA-4**
Denditric cell	**DC**
Helper T lymphocyte	**Th**
Interferon-γ	**IFN-γ**
Intercellular cell adhesion molecule-1	**ICAM-1**
Leukocyte function-associated antigen-1	**LFA-1**
Major Histocompatibility Complex	**MHC**
Microscopic Polyangiitis	**MPA**
Mucosal vascular cell adhesion molecule-1	**MAdCAM-1**
Myeloperoxidase	**MPO**
Proteinase 3	**PR3**
Renal Limited Disease	**RLD**
Soluble Fas	**sFas**
T regulatory cells	**Treg cells**
Tumor Necrosis Factor α	**TNF–α**
Tumor Necrosis Factor β	**TNF–β**
Vascular adhesion molecule-1	**VCAM-1**
Wegener Granulomatosis	**WG**

1 Summary

The aim of this thesis has been to study T cells in systemic vasculitis. The thesis consists of four papers addressing T cell activation markers, apoptosis and cell adhesion and migration.

In paper I: T cell activation in patients with ANCA-associated vasculitis: inefficient immune suppression by therapy

We studied the expression of T cell activation markers in patients with ANCA-associated vasculitis at onset of the disease, in remission and quiescence. These patients showed an increased expression of T cell activation markers irrespective of immunosuppressive therapy or disease phase.

In paper II: Serum sFas Levels Are Elevated in ANCA-positive Vasculitis compared with other Autoimmune Diseases

We determined levels of soluble Fas (sFas) in sera and Fas expression on mononuclear cells from patients with ANCA-positive vasculitis and other rheumatic diseases. The vasculitis patients showed high sFas levels and the levels remained elevated even in clinical remission.

In paper III: A CD26-Controlled Cell Surface Cascade for Regulation of T Cell Motility and Chemokine Signals.

We examined the influence of chemokines on the cell surface expression of endogenous thrombospondin-1(TSP-1) in T cells. The CD26-processed chemokines SDF-1α/CXCL12 and RANTES/CCL5, in contrast to a control chemokine not processed by CD26, were potent inducers of cell surface expression of TSP-1 and lipoprotein receptor related protein/CD91 through inhibition of a CD26-dependent suppressive mechanism.

In paper IV: Impaired CD26 suppression of cell surface Thrombospondin-1 expression in T lymphocytes from patients with autoimmune vasculitis

We studied patients with ANCA positive vasculitis with respect to serum levels of chemokines, T cell mRNA expression of chemokine receptors, and TSP-1 expression in comparison with patients with type 1 allergy and healthy controls. The results

demonstrated an increased cell surface expression of TSP-1 together with an impaired CD26 suppression in vasculitis patients suggesting that a mechanism for negative regulation of T cell migration is disturbed in these patients.

2 Introduction

2.1 The immune system

The major physiologic function of the immune system is to protect the body from damage caused by invading microorganisms and any toxic substance they may produce.

2.1.2 Innate immunity provides the first line of defence against microorganisms. It does not lead to immunologic memory but influences the adaptative immune response. The components of the innate immunity include the skin and internal epithelial layers, the movement of broncho-pulmonary cilia acting together with chemical factors such as lysozyme and phospholipase in tears, saliva, nasal secretion, defensins and gastric secretions. The normal flora of the skin and gastrointestinal tract may also prevent the colonization of pathogenic bacteria by secreting toxic substances or by competing with pathogenic bacteria. Cells involved in innate immunity are **granulocytes**, including **neutrophils, eosinophils, monocytes/macrophages** and **natural killer cells (NK cells).**

2.1.3 Inflammation is commonly defined by pain, redness, heat and swelling. Cytokines, TNF, Il-1, IL-10, TGF-β, the complement system, manose-binding lectin together with chemokines and adhesion molecules regulate the inflammation process and guide circulating leukocytes to the site of infection.

2.1.4 Adaptative immunity, also called specific immunity, is characterized by an exquisite specificity for distinct molecules, memory and more vigorous responses to repeated exposures to the same pathogen. Two types of adaptive immune responses are recognized; **humoral immunity** mediated by antibodies, consisting of five major classes (IgM, IgG, IgA, IgD, and IgE) produced by **B lymphocytes,** and **cell mediated immunity** mediated by **T lymphocytes**.

Each B and T cell has a unique receptor protein which binds to a specific antigen. This receptor on B cells is a membrane-bound immune-globulin. After meeting its cognate antigen and getting a signal from a T cell, B cells may differentiate into an

antibody producing cell or into a memory B cell. T cells recognize short peptides from self or foreign antigens through the **T cell receptor (TCR),** a heterodimmer consisting of two polypeptide chains, α and β, covalently linked by disulfide bonds. The T cell co-receptor CD3 and the ζ chain form the TCR-complex and transduce the signals that lead to T cell activation. Naïve T cells are cells that have not yet encountered antigens and that after encounter of antigen will further differentiate into **memory T cells** . Cell mediated immunity promotes the destruction of intracellular microorganisms, inaccessible to antibodies, and/or kill the infected cells thus eliminating reservoirs of infection.

Antigen presenting cells (APCs) ingest, process and present antigenic fragments associated with **Major Histocompatibility Complex (MHC)** molecules to T cells which recognize this complex through the TCR**.** APCs present peptides associated with MHC class I molecules to CD8+ T cells and associated with MHC class II molecules to CD4+ T cells (1). Almost all nucleated cells express MHC class I molecules and therefore may act as APCs presenting internal antigens while the expression of MHC class II is confined to a few cell types presenting external antigens. The main type of APC is the **dendritic cell (DC)** present in most organs where they capture the foreign antigens and transport them to the peripheral lymphoid organs. Other APCs are macrophages and B cells. **The immunological synapse**, consists of a central cluster of TCR molecules surrounded by a ring of adhesion molecules (2, 3). T cells also express other accessory molecules that do not recognize antigens but deliver signals to the T cells. The main accessory molecules on T cells are CD2, CD4, CD8, CD28, CD44, CD45R, cytotoxic T lymphocyte-associated protein-4 (CTLA-4 /CD152), leukocyte function-associated antigen-1 (LFA-1) and L-selectin. **CD26** is a 110 kDa widely distributed cell surface glycoprotein with intrinsic dipeptidyl peptidase IV(DPPIV) activity(4, 5) preferentially expressed on CD4+ CD45RO+ memory T cells and up-regulated after T cell activation. CD26 is a co-stimulatory protein for T cells involved in the signal transduction process and in the regulation of T cell maturation and cytokine secretion (5, 6).

T helper (Th) cells express a surface protein called CD4 and are presented peptide antigens associated with MHC class II molecules on the surface of APCs. They stimulate B cell growth and differentiation and activate macrophages to destroy phagocyted microbes by cytokine secretion. Upon antigenic stimulation naïve CD4+ T cells differentiate into effector subsets known as Th1, Th2 and Th17 characterized

by different functions and cytokine production. Th1 cells produce interferon-γ (IFN-γ) and lymphotaxin (LT), also called tumor necrosis factor–β(TNF-β), and play an important role in the elimination of intracellular pathogens and are associated with autoimmune diseases. Th2 cells secrete Il-4, Il-13 and Il-25, combat extracellular pathogens and are associated with allergy and asthma. Il-23 induces an expansion of Il-17 producing T cells that are important in the elimination of extracellular pathogens (7, 8). **Cytotoxic or Cytolytic T cells (CTLs)** express a glycoprotein called CD8 on their surface and recognize peptide antigens associated with MHC class I molecules on the APCs (9, 10). They kill tumor cells and cells infected with microbes, thus eliminating reservoirs of infection and are important in rejection of allografts.

Cytokines are peptides or glycopeptides secreted by almost all nucleated cells that act as intercellular messengers by stimulating their target cells to mature, differentiate, proliferate or undergo apoptosis. Due to their ability to mediate communication among leukocytes some cytokines are referred to as **interleukin**s. The majority of interleukins are synthesized by CD4+ T cells, monocytes, macrophages, and endothelial cells. **Interleukin-2 (IL-2*)*** is a 15- 18 kDa glycoprotein synthesized and secreted primarily by activated CD4+ T cells. It acts as a growth factor for T, B and NK cells, induces Th1 and Th2 effector T cell differentiation and supports development, survival and function of regulatory T cells (Treg cells)(11-14). **The interleukin 2-receptor (IL-2R)** is composed of three polypeptide chains, α, β and γ. The IL-2Rα chain (Tac or CD25) is expressed at low levels on circulating non activated lymphocytes and up-regulated after activation. The IL-2Rβ chain is constitutively expressed on NK cells and in low levels on resting T cells. The IL-2Rγ chain is constitutively expressed on resting cells and NK cells.

2.1.5 T cell motility and adhesion. Most mature lymphocytes recirculate continuously between blood and tissues. Naïve T cells are programmed to migrate to lymph nodes, Peyer´s patches, spleen and tonsils. Memory and effector T cells also traffic through lymphoid organs, intestinal lamina propia, pulmonary interstitium, joints and inflammed skin. The capacity to pass through the endothelial cells of the blood vessel wall (extravasation) (15, 16) at defined sites and to migrate within tissues controls the access of lymphocytes to particular organs. In areas of inflammation the endothelium recruits cells through elevated expression of vascular

adhesion molecule-1 (VCAM-1), intercellular cell adhesion molecule-1 (ICAM-1), mucosal vascular cell adhesion molecule-1 (MAdCAM-1), P and E-selectin**.** The process of extravasation is initiated through L-selectin mediated rolling of lymphocytes along the vessel wall (15). Stromal cell-derived factor-1 (SDF-1α/CXCL12) triggers arrest and firm αLβ2 and α4β1 integrin-dependent adhesion to the endothelial ligands ICAM-1 and VCAM-1 respectively, accompanied by transendothelial migration of lymphocytes to the extravascular sites (17, 18). The passage of the lymphocytes through the endothelium and basement membranes is followed by migration within tissues through interaction with extracellular matrix components (ECM) such as fibronectin, laminin and collagens.

TSP-1 is a matricellular glycoprotein with a molecular mass of about 450 kDa often found in tissues undergoing regeneration and up-regulated in proliferating cells (19, 20). TSP-1 is also expressed by T lymphocytes where it has shown to be involved in regulation of adhesion and migration (21-23). T cell TSP-1 is associated with **calreticulin (CRT),**(21, 24) a calcium-binding protein in the endoplasmic reticulum found at the surface of many cell types (24, 25) and **LRP/CD91** a large cell-surface glycoprotein expressed by most cell types (26) and with **CD47** an integrin associated protein expressed on the surface of all mammalian cells. A role of TSP -1 for the function of the immune system is suggested by the fact that TSP-1 gene knockout mice show early onset of pneumonia, increase in circulating monocytes and lymphocytes, impaired wound healing and inflammatory changes in many organs (27, 28).

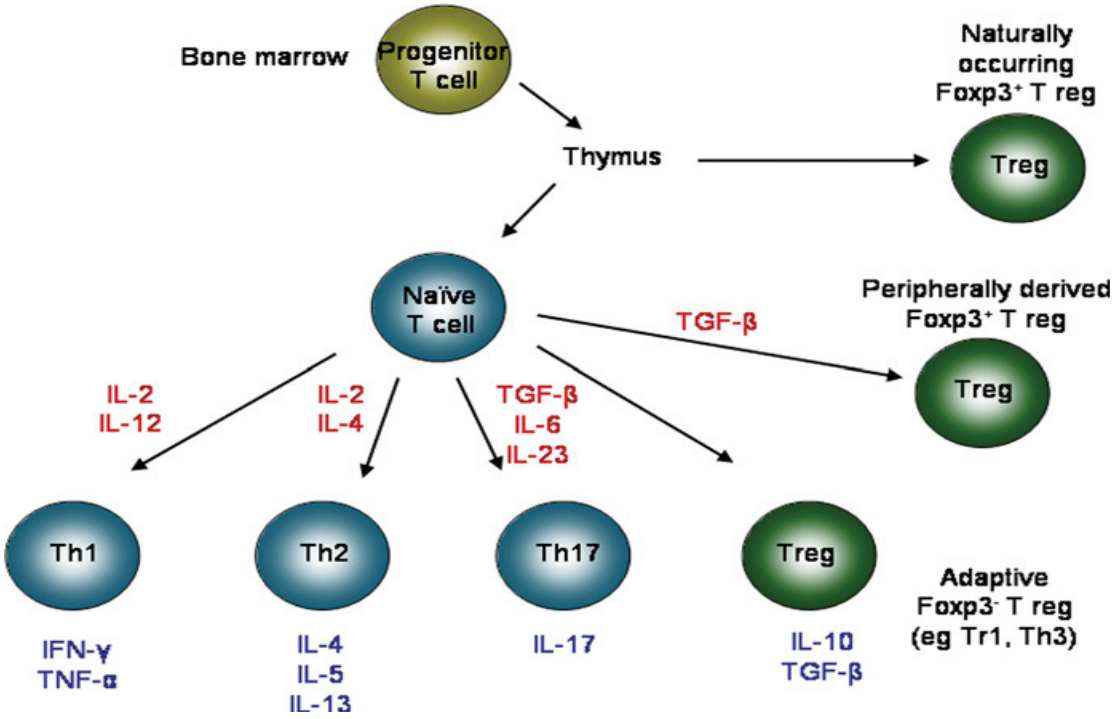
2.1.6 Autoimmunity

When specific lymphocytes meet antigens they may undergo activation resulting in an immune response or inactivation or elimination resulting in tolerance. Tolerance may be central censoring B and T cells while they differentiate in bone marrow and thymus or peripheral acting on mature recirculating B and T cells (29, 30). T and B lymphocytes with high avidity for self antigens are eliminated or negatively selected during maturation in the thymus and the bone marrow. However, some self reactive mature lymphocytes are found in all normal individuals. These self reactive lymphocytes remain anergic or are eliminated by **apoptosis** preventing breaking of tolerance and development of autoimmune disease (31, 32)

Autoimmunity can be defined as a state in which the mechanisms regulating self-tolerance fail and the immune system reacts to self-antigens. Autoimmune responses without autoimmune disease are not rare and may be important in maintaining a normal immune system (33-36). This natural autoimmunity is probably controlled during an immune response to prevent the development of high-affinity anti-self antibodies (37). Many healthy individuals thus have autoantibodies which bind to self antigens (35, 38, 39). Autoantibodies to a variety of serum proteins, cell surface and intracellular structures(38, 39) are present in newborns as well as in " germ-free" mice fed on an antigen free diet (40) and are thought to arise without antigen stimulation.

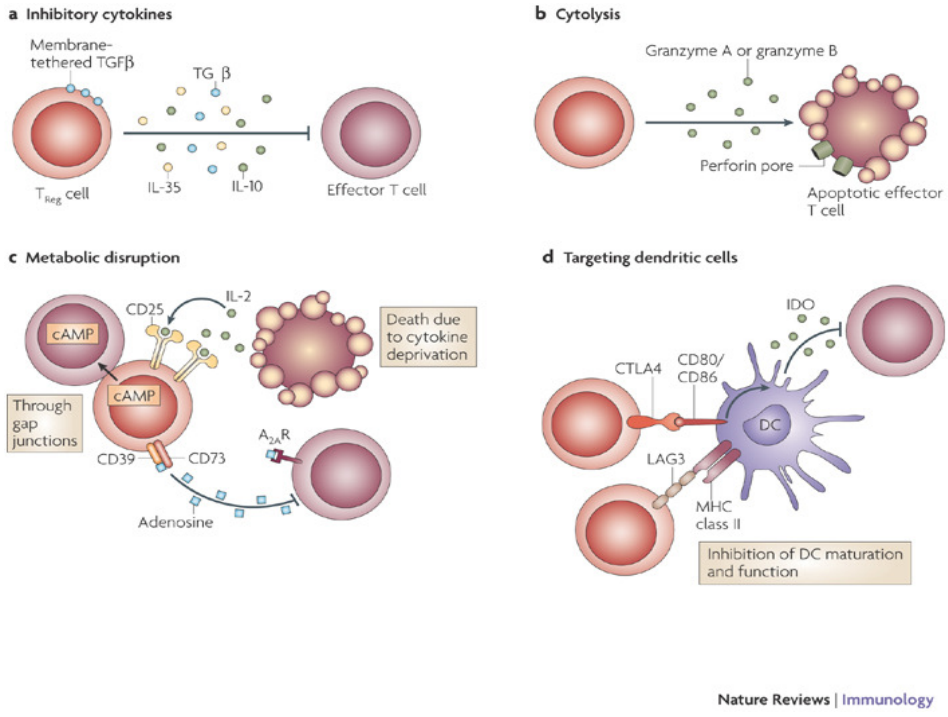
Autoimmune diseases develop when the adaptive immune responses are directed toward self-antigens leading to release and alteration of tissue antigens and activation of lymphocytes specific for those antigens(9, 41). These responses may initiate or perpetuate inflammatory reactions with leukocyte infiltration, tissue damage and fibrosis. Most autoimmune diseases are polygenic in nature (42-49) and both genetic and environmental factors are well documented contributors to autoimmunity (50-52).T cells have an important role in the pathogenesis of autoimmune diseases. In RA, SLE and celiac disease (CD), among other autoimmune diseases, T cells infiltrate and damage tissues(53, 54). In glomerulonephritis CD4+ T cells can mediate glomerular immunopathology through cytokine secretion or enhancement of auto-antibody formation (55). Defects in T cell homeostasis can result in T cell lymphopenia inducing homeostatic proliferation that subsequently may expand the pool of autoreactive T cells giving rise to autoimmunity (56-58).

Regulatory T cells (Treg cells) are immunosuppressive and limit chronic inflammatory diseases and antitumour immunity, and have been implicated in the control of autoimmune responses (59-66). Deficiency in the number of Treg cells or in their function can lead to autoimmunity, allergy and transplantation failure (60, 64, 67-74). CD25 and the forkhead box p3 (FOXP3 have been defined as markers of T reg cells(74). Different types of Treg cells have been described including CD4+CD25+ naturally arising Treg cells, interleukin 10 (IL-10) secreting TR1 cells, transforming Growth Factor-β (TGF-β) secreting Th3-cells, CD8+CD28- T-cells, CD8+ CD122+ T cells, γδ- T-cells and NK T-cells(30, 70, 75, 76)



Naturally occurring CD4+Foxp3+ Treg-cells are part of a diverse group of CD4+ subsets which also include effector Th1, Th2 and Th17 lineages. The cytokines in red are key molecules in the development of Th1, Th2, Th17 and Treg-cell (Treg) lineages. The cytokines in blue are some of the key molecules produced by the variousCD4+ lineages. R. J. Mellanby, D. C.Thomas and J.Lam

Functionally their suppressor activity can be grouped in four basic modes of action; by inhibitory cytokines, by cytolysis, by modulation of dendritic cells maturation or function and by metabolic disruption (74).



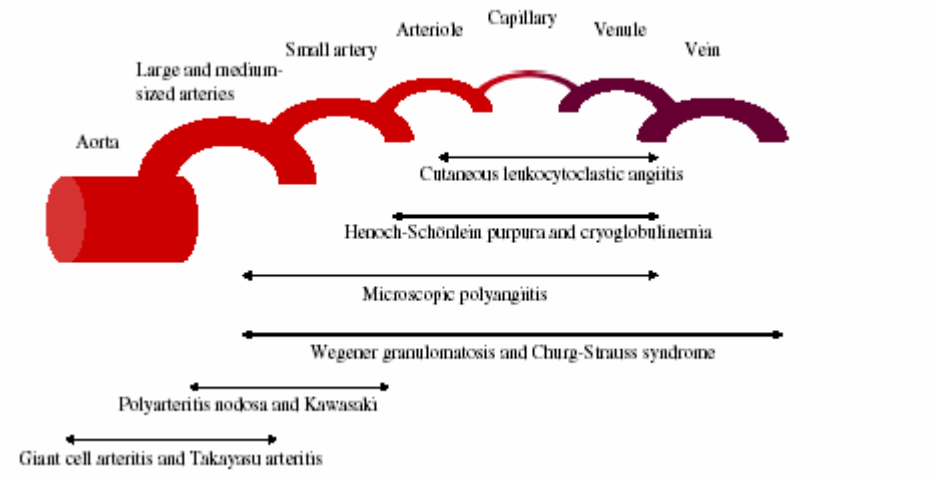
Description of the various regulatory T (TReg)-cell mechanisms centred around four basic modes of action. a | Inhibitory cytokines include interleukin-10 (IL-10), IL-35 and transforming growth factor- (TGF). b | Cytolysis includes granzyme-A- and granzyme-B-dependent and perforin-dependent killing mechanisms. c | Metabolic disruption includes high-affinity CD25 (also known as IL-2 receptor)-dependent cytokine-deprivation-mediated apoptosis, cyclic AMP (cAMP)-mediated inhibition, and CD39- and/or CD73-generated, adenosine receptor 2A (A2AR)-mediated immunosuppression. d | Targeting dendritic cells (DCs) includes mechanisms that modulate DC maturation and/or function such as lymphocyte-activation gene 3 (LAG3; also known as CD223)–MHC-class-II-mediated suppression of DC maturation, and cytotoxic T-lymphocyte antigen-4 (CTLA4)–CD80/CD86-mediated induction of indoleamine 2,3-dioxygenase (IDO), which is an immunosuppressive molecule made by DCs.
Vignalli et al 2008

The role of T reg cells has been extensively studied in autoimmune diseases. Inoculation of CD4+ T cells depleted of CD25+ cells in T-cell deficient BALB/c mice causes a variety of autoimmune diseases such as gastritis, insulinitis and thyroiditis all which could be prevented by transferring CD+CD25+ T cells (77). Studies in humans have shown that the proportion of CD4+ CD25+ T cells is lower in patients with newly diagnosed and long-standing Diabetes type 1 (78) and decreased in number in patients with clinically active systemic lupus erythematosus compared with patients in remission and healthy controls (62). Kriegel et al have shown that the suppressive capacity of CD4+CD25+ T cells is impaired (64) in patients with autoimmune polyglandular syndrome type II (APS-II) and studies in rheumatoid arthritis show that T reg cells are functionally compromised (63) and fail to suppress pro-inflammatory cytokine secretion in activated T cells and monocytes. In patients with myasthenia gravis alteration in both number and function of T reg cells have been reported (79).

2.1.7 Systemic vasculitis

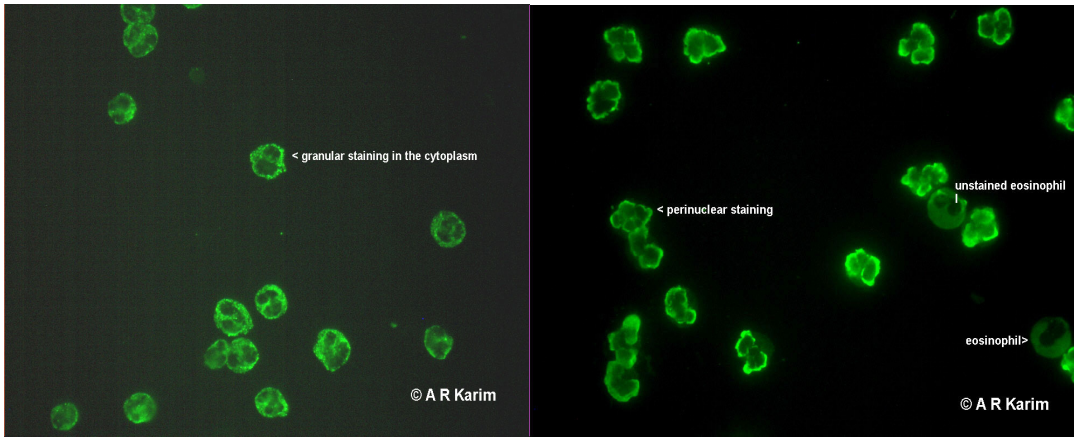
Vasculitis is defined as inflammation of blood vessels which leads to narrowing, often occlusion and subsequently to infarction and tissue damage. It may be a primary or idiopathic process of unknown aetiology or secondary to other pathological processes such as infections, malignancies or inflammatory diseases (80-82) Vasculitis can also be classified according to the size of blood vessel involvement and as limited or systemic, affecting various organs at the same time. The primary

systemic vasculitides are heterogeneous multi-system disorders affecting the small and medium blood vessels(83).



2.1.8 Anti-neutrophil cytoplasmic antibodies (ANCA)

The presence of anti-neutrophil cytoplasmic antibodies (ANCA) has been a great aid in the diagnosis of systemic vasculitis (84-86). ANCA has been found to be associated with four vasculitides: Wegener´s granulomatosis (WG), microscopic polyangiitis (MPA), Churg-Strauss syndrome and necrotizing crescentic glomerulonephritis (84, 86, 87). ANCA are mainly directed against two main antigens, proteinase 3 (PR3) and myeloperoxidase (MPO) and give a cytoplasmic or perinuclear immunofluorescence pattern respectively. **PR3**(88) is a 29-32 kDa neutral serine proteinase present in the α–granula of neutrophils, monocytes and basophils (88-92).



Anti- PR3 antibodies are present in nearly 80% of patients with WG. **MPO** is a 140-kDa heme-containing homodimer preferentially present in polymorphonuclear neutrophils (PMN) (93, 94). Anti-MPO antibodies are present in nearly 70% of patients with MPA.

ANCA associated vasculitis occurs at any age. The incidence of WG and MPA has been shown to vary in different parts of Europe. In Tromsö (Norway) the incidence of WG is 10,5/million and the incidence of MPA 2,7/million, in Norwich (England) it is 10,6/million for WG and 8,4/million for MPA while in Lugo (Spain) it is 4,9/ million and 11,6/million respectively (95). The incidence of pauci-immune necrotizing and crescentic glomerulonephritis in Stockholm 1995 was 3.9/100000 population in patients over 65 years (96). In two studies in USA caucasians comprised more than 90% of cases with WG while 1-4% were Afroamericans, Hispanics and Asians (97).

The role of ANCA in the pathogenesis of autoimmune vasculitis has been extensively studied (98-102). ANCA influence neutrophils and monocytes causing cell damage, activation, degranulation and release of chemoattractants. (84, 103). ANCA prevent the inactivation of PR3 by its natural inhibitor, α1-antitrypsin and α1-antitrypsin deficiency has been associated with an increased risk of PR3 associated vasculitis(104, 105). ANCA may increase adhesion of neutrophils to endothelial cells and induce apoptosis of endothelial cells and neutrophils(106, 107). ANCA may further opsonise neutrophils, thus accelerating their phagocytosis by macrophages with subsequent release of IL-1, IL-8 and TNF-α and stimulation of inflammation(100, 107, 108).

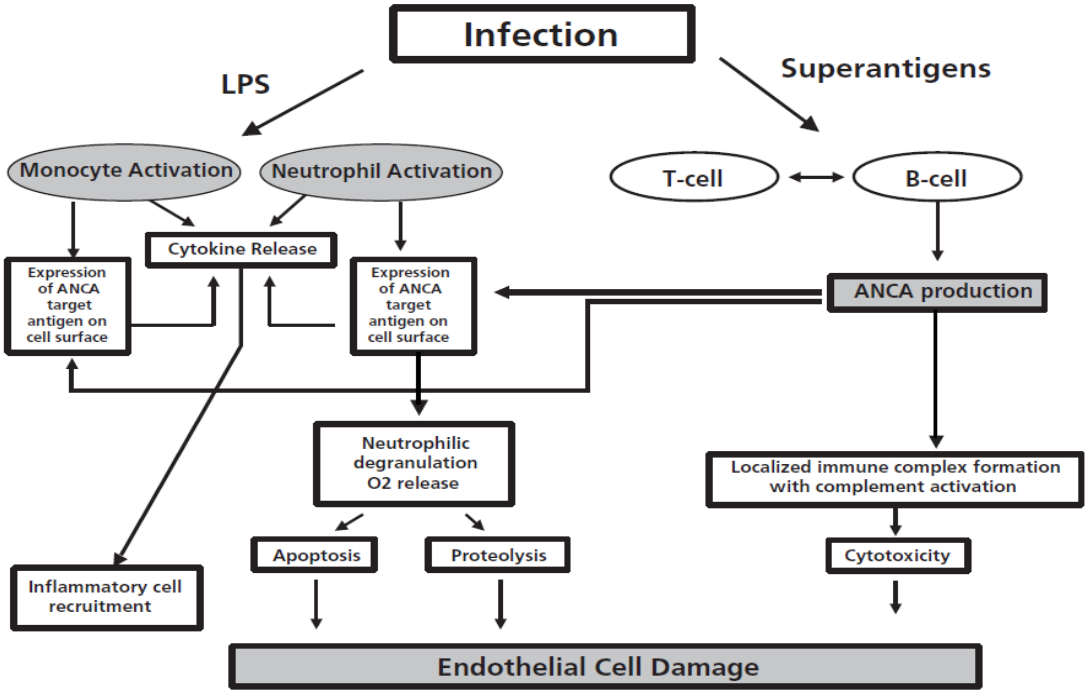


Figure 1. Schematic concept of proinflammatory effects of ANCA leading to vasculitis. Cofactors such as infections can trigger an inflammatory response leading to cytokine-mediated priming of monocytes and neutrophils. This results in cell surface expression of ANCA target antigens (such as PR3), which then may become a target for circulating ANCA. ANCA production may be the result of interactions between T cells and B cells, activated by microbial superantigens. Stimulation of primed neutrophils and monocytes by ANCA induces degranulation with protease release, and a respiratory burst with release of radical oxygen species. These effects result in direct injury to endothelial cells. Invoked mechanisms include cytotoxicity and apoptosis, but also localized immune complex formation with complement activation. LPS = lipopolysaccharide; ANCA = antineutrophil cytoplasmic antibodies.

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ANCA correlate poorly with disease activity, a rise in ANCA levels usually reflects a flare of the diasease(109, 110) but should be interpretated together with other laboratory parameters and clinical signs(99, 111). ANCA-negative Wegener´s patients usually do not develop a generalized form of the disease as long as they remain ANCA-negative(100).

2.1.9 Wegener Granulomatosis (WG)

WG is a granulomatous inflammation involving the respiratory tract with necrotizing vasculitis affecting small to medium-sized vessels (e.g, capillaries, venules, arterioles, and arteries) necrotizing glomerulonephritis being common (87). WG can occur at any age (average 40-55 years) (116) but the incidence increases with age being particularly high in the elderly. Over 90% of the patients present symptoms related to the upper or lower airways. Sinus and nasal inflammation can produce pain , patients may have epistaxis, nasal septum destruction with saddle nose deformity, persistent otitis media and hearing loss. Chest radiographic images can present as cavitating and non cavitating nodules or as ground-glass infiltrates. Patients may have hematuria, proteinuria and rapidly progressing renal failure. Renal histology shows a focal,

segmental necrotizing crescentic glomerulonephritis with little or no immune complex deposition. Other symptoms may be skin rash, migratory arthritis and ocular involvement. Patients have high risk for venous thrombosis despite no thrombophilic abnormalities. Mortality among untreated patients due to renal or/and pulmonary failure is approximately 80% while treated patients show a 5 year survival of 75%.

2.1.10 Microscopic polyangiitis (MPA)

MPA affects small vessels with necrotizing arteritis involving small and medium sized arteries (83). MPA is more common in males and lacks granuloma formation. Kidneys are involved in almost 79%, joints in 65- 72%, the gastrointestinal tract in 32-58% , lungs in 12-29% and the skin in 44-58%. Necrotizing glomerulonephritis is common and often with pulmonary capillaritis.The estimated survival after 5 years is about 75% (112, 113) and relapses are seen in more than 35% of patients. In both WG and MPA clinical signs at onset are important for the prognosis. Impaired renal or cardiac function, gastrointestinal and/or neurological involvement are associated with a poor prognosis (114).

2.1.11 Renal limited disease (RLD) presents solely with necrotizing crescentic glomerulonephritis (83, 115)

Treatment of these systemic vasculitides usually involves the use of medications that suppress parts of the immune system(113, 116, 117). New trials, including TNF–α blockade , T and B cell depletion and CD28 co stimulatory signal blockade, have been and are being evaluated(118-120). Early death in patients with systemic vasculitis has been shown to be due to adverse events rather than disease activity (121).

2.1.12 T cells in systemic vasculitis

Cellular immunity has been shown to have a major role in systemic vasculitis associated with PR3 and characterised by granulomatous inflammation and defective T reg cells whereas the role of cellular immunity in MPO associated vasculitis is less clear(122).

The total lymphocyte count and absolute and relative numbers of CD4+ T cells are lower in patients with vasculitis compared with healthy controls (123-125) and the

percentage of CD8+ T cells are increased (125) with a low CD4/CD8 ratio particularly lower in patients with renal involvement and long-lasting severe disease(126). T cell surface expression of markers of activation is higher than in healthy controls and remains high even during remission. The soluble levels of T cell activation markers such as sCD25 and sCD30 are also higher and high levels are associated with persistent ANCA positivity (125) (127). An expansion in the population of CD4+ effector memory cells (CD4+T_{EM}) has been demonstrated in WG(128). These cells can migrate to sites of inflammation and produce large amounts of inflammatory cytokines. They require a persistent immune trigger supporting the presence of ongoing antigenic stimulation(128, 129).Vogt et al detected short telomeres in addition to telomerers of normal length in patients with disease for more than 5 years, indicating replicative senescence of some T-cell clones as a consequence of activation (130). WG patients show up-regulation of CTLA-4 protein on T cells(131) which may signify resistance to apoptosis(132) and further show high serum levels of sFas even in clinical remission (133) indicating a persisting chronic immune activation. Soluble Fas (sFas) has been suggested to protect from Fas-mediated apoptosis(134).

Th1 type responses have been implicated in the development of the granulomatous lesions in WG and a "switch" to Th2 type responses together with a less Th1 type cytokine production has been observed in the progression towards more generalized disease with vasculitic manifestations (136, 137. It has recently been demonstrated that superantigens and peptidoglycans from Staphylococcus aureus increase the risk of relapse with an overproduction of IL-17 by Th17 cells with a strong inflammatory response contributing to granuloma formation, antibody production and tissue injury in patients with WG(135).The IgG subclass pattern of ANCA with a predominance of the IgG1 and IgG4 subclasses, indicates a T cell-dependent immune response(136).

3 Aims of the studies

The aim of the studies has been to investigate the role of T cells in ANCA-associated vasculitis.

Paper I

We examined T cell activation markers both at onset of the disease, in remission and in quiescence.

Paper II

We studied sFas/sFasL levels at onset of the disease, in clinical remission and in quiescence.

Paper III

We studied the influence of CD26/DPPIV, TSP-1 and chemokines on the regulation of T cell motility.

Paper IV

Based on the results in paper III we focused on the possible role of a CD26-controlled chemokine-TSP-1-CD91 cascade in patients with ANCA-associated vasculitis.

4 Material and Methods

4.1 Material

Paper I

Peripheral blood cells (PBC) and serum samples from 9 patients with WG, 9 with cWG (clinical WG), 5 patients with MPA, 4 patients with RLD and 11 healthy controls (HC) were analysed with respect to cell surface activation markers using flow cytometry and serum levels of IL-2r using ELISA. Patients were categorised with respect to the phase of their disease in three groups. Group 1a: 16 patients with newly diagnosed active disease**.** Group 1b: 10 of the patients from group 1a were reanalysed 3 -11 months later in first clinical remission. Group 2, 11 patients in quiescence.

Paper II

Serum samples from 37 patients with ANCA-positive vasculitis, 29 of them with newly diagnosed active disease (17 of them reanalyzed in first clinical remission) and 12 in quiescence, 29 patients with rheumatoid arthritis (RA), 14 patients with systemic lupus erythematosus (SLE), 10 patients with Sjögren´s syndrome (SS), 7 stable patients on dialysis and 26 healthy controls were analysed with respect to serum levels of sFas and sFasL using ELISA. Peripheral blood cells (PBC) from 6 patients with ANCA-positive vasculitis at onset of the disease and after initial treatment, 7 patients on dialysis without inflammatory/autoimmune disease and 6 healthy controls were studied by flow cytometry with respect to the cell surface expression of CD95(Fas). PBC from 6 patients with ANCA-positive vasculitis at onset and after initial treatment were studied with respect to the expression of Fas in mononuclear cells by RT-PCR.

Paper III

Human lymphocytes and a specific T cell clone were cultured as described in the paper and examined with respect to the influence of chemokines and endogenous TSP-1 on T cell adhesion and migration.

Paper IV

Serum samples from 20 patients with WG, 1 patient with localized WG (LWG), 9 with MPA, 1 with RLD and 18 healthy controls were analysed with respect to serum levels

of chemokines with ELISA. PBT from 8 patients with WG, 3 with LWG, 3 with MPA and 2 with RLD at onset were studied by RT-PCR with respect to the gene expression of chemokines and 6 of them reanalysed after treatment. PBT from 7 patients with WG, 1 with RLD, 1 with MPA, 7 patients with allergy and 9 healthy controls were studied with respect to cell surface expression of TSP-1 and the inhibitory effect of CD26 on TSP-1 by quantitative immunocytochemistry.

4.2 Methods

A brief description of the method used in the discussed papers.

Flow cytometry (FACS)

Three-colour immunofluorescence analysis was performed according to standard procedures for flow cytometry of whole blood samples, using a "lyse & wash" protocol. EDTA blood was mixed with appropriate concentrations of directly fluorochrome-conjugated monoclonal antibodies; the lymphocyte/monocyte markers CD45/14 and antigens on T cells (CD3, CD4, CD5, CD8), B cells (CD20) and NK-cells (CD56/16).The following molecules associated with cellular activation were included; IL2 receptor (CD25), T cell co-receptor (CD28), CD38, CD69, HLA-DR and antibodies to Fas/CD95 (APO1). Antibodies to detect CD45RA and CD45RO-positive T cells were used to define"naive" and "memory "T cell subsets, respectively. All antibodies were obtained from Becton-Dickinson (BD), Mountain View, CA, USA). Flow cytometry was performed on a FACScan (BD) using the Cellquest software (BD), both for acquisition and analysis. All samples were analysed by setting appropriate SSC/FSC gates around the lymphocyte population, using back-gating on CD45 positive/ CD14 negative cells. Consistency of analysis parameters was ascertained by calibrating the flow cytometer with Calibrite beads and the AutoComp software, both from BD.

RNA extraction and RT-PCR for Fas

Total RNA was isolated from PBMC using the guanidinium thiocyanate-phenol chloroform method. The RNA was treated with Rnase inhibitor(Rnasin, Promega, Madison, USA), 1 µL/50 µL RNA and stored at -70ºC if not used immediately. Single stranded cDNA copies were made from 1 µg of total RNA using random hexamers and murine leukemia virus transcriptase (Perkin-Elmer, Roche Molecular

Systems, Inc., NJ, USA). RT-PCR was performed at 42ºC for 15 minutes followed by denaturation at 99ºC for 5 minutes. The following primers were used:

Fas receptor:	5'-TCTTTCACTTCGGAGGATTGCT-3'
	5'-ACTTTCTGTTCTGCTGTGTCTT-3'
ß-actin:	5'-GTGGGGCGCCCAGGCACCA-3'
	5'-CTCCTTAATGTCACGCACGATTTC-3'

Twenty µL of the cDNA reaction mixture and the specific primers were used with standard protocol to amplify the 832 bp (Fas) and 540 bp ß-actin fragments. PCR products were analyzed on 1.5% agarose gels, stained with ethidium bromide and analyzed under UV light.

Immunohistochemistry

Paraffin sections of kidney biopsies were analyzed immunohistochemically for Fas and FasL expression. MoAbs were used: Fas (CD95, clones APO-1 and DX2, Dakopatts, Glostrup, Denmark) and Fas Ligand (CD95L, clone 5D1, NovoCastra Lab, Newcastle, UK). The endogenous peroxidase activity was blocked with hydrogen peroxide (3%). A heat induced antigen retrieval process was used before staining for Fas and CD95L. The immunohistochemical staining, a streptavidin-biotin procedure, was performed in a DAKO TechMate® 500 PLUS, using DAKO ChemMate® solutions (Dakopatts).

Enzyme –linked immunorbent assay (ELISA)

Serum levels of sFas and FasL and chemokines (CCL4, CXCL12, CCL5, CCL3, CXCL8, CCL17 and CXCL10) were assessed using kits from R&D systems (Minneapolis,MN, USA) according to the protocol recommended by the producer. In brief the method consists of;

1. attaching specific antibodies to the plastic plates.
2. addition of test solution containing antigen.
3. addition of enzyme-labelled antibody, incubated according to the manufactures instructions.

4. addition of enzyme substrate and measurement of coloration of the plates.
5. washes to remove excess non-bound reactants

RNA Extraction and Reverse Transcription, chemokines

Total RNA was isolated by centrifugation (400 g for 10 minutes) using the QIAAMP RNA blood Mini-Kit(Qiagen, Hilden, Germany). Six milliliters of red cell lysis buffer was used for 1 mL of buffy coat. Total RNA was extracted from the cell lysate according to the manufacturer's instructions. Complementary DNA(cDNA) was synthesized in a total volume of 50 µLcontaining 30 µL of RNA, 1µ first-strand buffer (supplied with reverse transcriptase [RT]), random hexameroligonucleotide primer (105 µg/mL; Pharmacia Biotech, Uppsala, Sweden), deoxynucleoside triphosphates(1 mmol/L of each; Pharmacia Biotech), dithiothreitol (1 mmol/L; supplied with RT), RNasin (0.5 U/µL; Promega, Madison, WI), and Moloney murine leukemia virus RT (4.8 U/µL; Invitrogen, Paisley, Scotland) at 37°C for 1.5 hours. The reaction was terminated after 15 minutes at 68°C, and cDNA was stored at -20°C pending further use.

Real-Time Quantitative polymerase chain reaction (RT-PCR) quantification of cDNA was performed with an ABI Prism 7000 Sequence Detection System (Applied Biosystems, Foster City, CA). Primers and probes for CCR5, CXCR3, CCR1, CCR2, and glucose-6-phosphate dehydrogenase (G6PD) were selected using Primer Express software version 2.0 (Applied Biosystems). The TaqMan probes contain the fluorophores 5=FAM as a reporter and 3=TAMRA as a quencher. Each set of TaqMan primers/ probes was designed to target cDNA alone without contaminating DNA. Primer sets and probes were commercially synthesized by CyberGene (Huddinge, Sweden). PCR reactions were performed in duplicate in 96-well optical plates in a total volume of 25 µL.Each reaction contained 2.5 µL of cDNA, 12.5 µL of TaqMan Universal Master Mix (containing deoxynucleoside triphosphates with deoxyuridine triphosphate), MgCl2, DNA polymerase (AmpTaq Gold), Passive Reference (ROX) (Applied Biosystems), 300 nmol/L of each TaqMan primer, and 200 nmol/L of TaqMan probe. Positive and negative controls were included in all runs. Thermal cycling conditions were 2 minutes at 50°C initially and 10 minutes at 95°C. Cycle conditions were 40 cycles at 95°C for 15 seconds and at 60°C for 1

minute. The housekeeping gene G6PD was included as an endogenous normalization control, which was used to confirm successful RNA isolation and RT and the total amount of RNA in every sample. To calculate the relative gene expression, Ct method (User Bulletin 2, ABI Prism 7700 SDS, Applied Biosystems) was used. With this method, results were calculated as the ratio between the gene of interest (CCR5,CXCR3, CCR1, and CCR2) and the endogenous control in the same sample. The mRNA expression levels were expressed as the -fold increase of chemokine receptor expression level normalized to G6PD. The intra-assay coefficient of variation for this method was <10% for all markers. Throughout the study, a 2-fold increase was accepted as a significant increase. In each sample, transcription levels were compared with the microbiologic and histologic diagnosis established by conventional methods.

Cell Culture

Human lymphocytes were purified using Lymphoprep density gradient separation followed by treatment with carbonyl iron and magnetic removal of phagocytic cells and stimulated with anti-CD3 in the presence of IL-2 and IL-4 for 3-5 days before the experiments. The birch (Bet v 1) specific T cell clone AF 24 was regularly stimulated with anti-CD3 and cultured in the presence of IL-2 and IL-4 for 5-12 days before the experiments. All cells were cultured in RPMI 1640(Gibco Ltd, Scotland) supplemented with 2 mM L-glutamine, 0,16 % sodium bicarbonate, 10000 U/ml benzyl penicillin, 100000 µg/ml streptomycin and 10 % FCS or in serum-free AIM V medium. To induce down-modulation of CD26 the lymphocytes were incubated with anti-CD26 (TA5.9) for 4 hours.

siRNA-mediated CD26 silencing

The expression of CD26 was suppressed using the human T cell Nucleofector kit (Lonza, Köln, Germany) and a Nucleofector device (Amaxa biosystems, Köln, Germany). CD26-siRNA (h) (sc-42762) and control-siRNA (sc-37007) were obtained from Santa Cruz Biotechnology (CA, USA).

Biotinylation and Immunoprecipitation of Cell Surface Proteins

Anti CD3 activated cells were biotinylated in solution using reagents for biotinylation from Cellular Labeling and Immunprecipitation Kit, Roche. The cells were allowed to

adhere for 30 min. Where stated, cells were treated with 4N1K (50 μM) , scrambled 4N1K (Sc4N1K) (50 μM), Diprotin A (50 μM) or the chemokines CXCL12 (50 ng/ml) and CLL5 (50 ng/ml) for 30 min before biotinylation. Adherent cells were washed and biotinylated while remaining attached (0,5 ml biotinylation buffer/well). Adherent cells were scraped off the plate, washed, lysed and centrifuged as above. For pre-clearing, each tube with lysate was incubated with protein G agarose at 4°C over night. Agarose beads were discarded and lysates mixed with 1 μg of TSP-1 antibody or CD91 antibody and incubated at 4°C over night. Protein G agarose was added to each tube followed by incubation at 4°C over night. Beads were washed and 20 μl reducing sample buffer was added followed by boiling for 4 min. Proteins were separated on 6% SDS-PAGE gels.

Quantitative Immunocytochemistry

Glass chambers were coated with ICAM-1 (2 μg/ml) or fibronectin (10 μg/ml) overnight at 4°C and washed. Anti-CD3 activated cells in serum-free AIM-V medium were allowed to adhere to the coated surface for 30 min at 37°C followed by fixation with 2% paraformaldehyde at 4°C for 20 min. For detection of CD26 the cells were subsequently permeabilized using 0.1% saponin. Mouse anti-human antibodies to TSP-1, CD91 or CD26 were added, followed by detection of the primary antibody with the biotin/avidin/HRP based Vectastain ABC-Kit (Vector Laboratories) and a peroxidase substrate (3-amino 9-ethyl carbazole). Pictures were taken of each slide using the program NIKON ACT-1 and a NIKON Eclipse E1000M microscope at 600x magnification. Mean staining intensity (arbitrary units) was determined using the Image J picture analysis program.

Cell migration

Collagen type 1 was diluted in serum-free RPMI 1640 and H_2O (8/1/1), applied in plastic Petri-dishes 1ml/dish (30 mm; BD Biosciences) and allowed to polymerize at room temperature. Chemokines were dissolved in the gel, while antibodies and peptides were present with the cells. A total of $1.0x10^6$ cells in AIM-V medium was added to each well and allowed to migrate for different times. The cells were fixed in 2.5 % glutaraldehyde and washed twice with PBS. Migration was evaluated in nine fixed positions in each well and at 50 μm intervals throughout the gel by the use of an inverted microscope (Nikon Eclipse TE300) and a digital depth meter (Heidenheim

ND221). The infiltrating cells were identified in situ in the collagen gels using immunocytochemistry after fixation in paraformaldehyde.

Cell adhesion

To study cell adhesion, plastic Petri dishes (90 mm. Heger A/S, Norway) were coated with ICAM-1 (2 μg/ml) or fibronectin (10 μg/ml) and extensively washed before use. The cells (10,000/position) in AIM-V medium were incubated on the substrates in a humidified CO_2 incubator at 37(C for 15 or 30 min. Cells were fixed in 2.4 % cold glutaraldehyde (GTA) for 10 min and unbound cells were removed by gentle aspiration. The number of adherent cells per microscopic field (20 x objective) was counted in five positions. For immunocytochemistry, 2 % paraformaldehyde was used, and washed twice with PBS. Cell adhesion was routinely, unless otherwise stated, evaluated in five fixed positions.

Statistical Analysis

The Mann-Whitney U-test and The Wilcoxon rank sum-test were used to compare data between groups (the Kruskall-Wallis test was used first) was used to compare data between groups. P-values of <0.05 were considered significant. Analyses of paired observations of samples taken before and after therapy from the same individual were done, using matched-pair analysis of variance, corresponding to a paired t-test. Correlation was calculated by Spearman rank-order correlation. Staining intensity in immunocytochemistry experiments is presented as mean arbitrary units +- SEM. For analysis of adhesion and spreading, mean values +/- SD is shown. For determination of differences between inhibitor-treated lymphocytes and control lymphocytes, paired student's t-test was used. A 2-tailed value of less than 0,05 was considered statistically significant.

5 Results and discussion

Cell mediated immunity has been implicated in the development of WG by several groups (124, 136, 137) and an up-regulation of markers for T cell activation, absent from or present at very low levels on the surfaces of resting cells, such as CD69, CD25, and HLA-DR has been described in autoimmune diseases (135, 136, 138-140). In paper I we studied patients with WG, MPA and RLD with respect to activations markers and compared patients at onset of the disease and not on treatment with the patients after first remission, with patients in quiescence and with a group of HC. Patients at onset of the disease showed significantly higher levels of CD45RA+ CD4+ (naïve) T-cells than the same patients after remission as well as higher levels than in quiescence. The expression of CD45RA on CD4+ T cells was down-regulated in all patients when compared with HC. Levels of soluble IL2r (sIL2r), the soluble form of the IL-2Rα chain (Tac or CD25), were significantly higher in patients at onset and in quiescence compared with HC. Patients at onset even showed significantly higher sIL2r levels than after first remission and in quiescence. We did not measure the expression of CD25 on CD4+ T cells but it is well known from other studies that the expression of CD25 on CD4+ T cells is increased in patients with ANCA associated vasculitis(140, 141). We studied the expression of CD25 on CD3+ T lymphocytes and the lack of significant up-regulation of CD25 on this population may partly reflect a lower percentage of CD4+ T cells(123). We also found a significant down regulation of CD28 on CD3 + T cells at onset and a lower expression, that was not significant, after first remission and in quiescence compared with HC as well as significantly increased expression of CD38 on CD8+ T cells at onset of the disease and in quiescence .

The CD45 isoform CD45 RA is preferentially expressed on naïve T lymphocytes (142, 143). In our studies the expression of CD45RA was decreased on CD4+ T lymphocytes suggesting that a continuous T cell activation enhances the “memory” CD45RA- T cell population. IL-2r is induced upon activation and not expressed on resting cells. In both SLE and WG increased levels of sIL2r have been used as serum markers of T cell activation (141, 144). The decreased expression of CD28 on CD3+ T cells appears to be associated with T cell cytolytic activity, an impaired response to co-stimulation and shorter telomere length (145, 146) which is associated with terminal T cell

differentiation as a consequence of activation of the immune system. These findings are therefore, compatible with a continuous state of T cell activation in these groups of patients. The increased expression of CD38 on CD8+ cells in autoimmune diseases may be explained by the role of CD38 in cell activation, (147-149) and its involvement in the adhesion between leukocytes and endothelial cells(150).Based upon the present findings of surface activation markers in vasculitis patients it seems correct to assume that T cells in ANCA positive vasculitis are activated and remain activated throughout the disease course despite therapy and clinical remission.

A large number of studies have reported disturbances of the apoptosis regulatory mechanisms in immune cells as a pivotal element in the pathogenesis and evolution of autoimmune disorders (151-153). Activated T cells up- regulate the expression of Fas ligand (FasL) which binds to the Fas-expressing cell to induce apoptosis(154). In ANCA associated vasculitis apoptosis of neutrophils is disrupted by anti-neutrophil cytoplasmic antibody activation resulting in abnormal removal of these cells promoting persistent inflammation. (155, 156). In paper II we studied the Fas/Fas ligand system in ANCA –vasculitis. Soluble Fas (sFas) levels were more increased in the vasculitis group compared with HC and interestingly the levels increased despite decreased disease activity. We compared patients with vasculitis with patients with SLE, RA and SS who also showed increased levels of sFas when compared with HC confirming previous studies on sFas levels in autoimmune diseases (157-159) The expression of Fas in glomeruli was not apparently increased in patients with vasculitis compared with patients with SLE and IgA nephropaties. This may be consistent with the chronicity of the disease as in patients after recovery of the renal function apoptosis of infiltrating and intrinsic cells in the inflamed tissue may be a mechanism for the clearance of the cells(160). The higher levels of sFas in our study are consistent with a disturbance in apoptosis leading to abnormalities in the regulation of T cell activation supporting the findings in paper I of persistent T cell activation in ANCA-associated vasculitis. High levels of soluble Fas secreted from cells may block the binding between membrane Fas and FasL and inhibit apoptosis of lymphocytes that consequently survive longer(161).These disturbances in apoptosis may account for the failure to terminate immune responses and control autoreactive lymphocytes (162).

Autoimmune diseases are often characterized by extensive T cell infiltration of the affected organs. Basic T cell functions, such as adhesion and migration and chemokine regulation of lymphocyte extravasation and migration within tissues are probably important for T cell infiltration of tissues during inflammatory conditions (163, 164). Endogenous TSP-1 is important in the regulation of T cell adhesion and migration, interacting with multiple cell surface receptors, including $\alpha 3\beta 1$, $\alpha 4\beta 1$, and CRT via its N-terminal domain, and with CD47 and lipoprotein receptor related protein/CD91 via its C-terminal domain (22). In paper III we investigated whether chemokines may cross-talk with TSP-1 and its cell surface receptors. The chemokine CXCL12 binds to the chemokine receptor CXCR4 which is associated with CD26. We found that the chemokines CXCL12 and CCL5 that are processed by CD26, are potent inducers of cell surface expression of TSP-1 in T cells through a CD26-controlled mechanism and that TSP-1 stimulates expression of CD91. Silencing or modulation of CD26 increased TSP-1 expression and enhanced cytoplasmic spreading and T cell migration indicating that CD26 is an inhibitor of TSP-1 expression and that chemokines stimulate cell polarity and migration through abrogation of the CD26 dependent inhibition of TSP-1 expression. These findings suggest that T lymphocyte motility is regulated by a cascade of interacting cell surface molecules.

Based on the findings in paper III we further studied (paper IV) the possible involvement of the CD26-controlled chemokine-TSP-1-CD91 cascade in T cell regulation in patients with the ANCA associated vasculitides WG, MPA and RLD and compared these with patients with chronic symptoms of asthma due to type 1 allergy. We found that patients with vasculitis, but not patients with asthma, exhibited a higher TSP-1 expression on the T lymphocyte surface than healthy controls and that the capacity of CD26 to suppress TSP-1 expression was impaired in patients with vasculitis. Patients with WG, MPA and RLD showed increased serum levels of CXCL12 and CCL4 together with decreased levels of CLL5 which is consistent with an antagonistic effect of chemokines on the CD26-dependent suppression of TSP-1 in autoimmune vasculitis. The elevated serum levels of CXCL12 and CCL4 in WG and MPA/RLD patients may reflect increased production in tissues and leakage to the vascular compartment due to infections or disturbances in chemokine metabolism. CCL4 and CXCL12 play a crucial role in viral and microbial infections and infections have been implicated in the pathogenesis of WG (165) The vasculitis

patients showed lower mRNA expression of CXCR3, a prognostic T cell marker and potential therapeutic, which further supports involvement of chemokines and their receptors in WG and associated vasculitides. The results point to the possibility that the CD26-controlled chemokine-activated TSP-1- CD91 cascade is disturbed in vasculitis patients as a consequence of overproduction of chemokines.

In conclusion, these results support the hypothesis that T cells play a pivotal role in ANCA associated vasculitis and that understanding their function may lead to the development of new and more specific therapeutic strategies.

6 Populärvetenskaplig Sammanfattning

Detta arbete fokuserar på T lymfocyternas roll i en grupp av kärlsjukdomar kallade systemiska vaskuliter eller ANCA-associerade vaskuliter. T lymfocyter är en typ av vita blodkroppar som är viktiga för infektionsförvaret men även för avstötning av transplantat och uppkomst av autoimmuna sjukdomar, dvs sjukdomar som kännetecknas av att kroppens immunförsvar angriper den egna organismen och orsakar skada i olika vävnader. Vi har studerat tre autoimmuna kärlsjukdomar: Wegener´s granulomatosis (WG), microscopisk polyangit (MPA) och ”renal limited disease” (RLD) som framför allt påverkar njurarna och de övre och nedre luftvägarna. I våra studier har vi kunnat påvisa att T lymfocyterna är aktiva under hela sjukdomsförloppet och klarlägga en del av de mekanismer som påverkar dessa cellers funktion. Resultaten antyder att terapi specifikt riktad mot T cellerna kan erbjuda bättre behandlingsresultat av systemiska vaskuliter.

7 Concluding Remarks

1. In patients with ANCA-positive vasculitis at onset, in remission and quiescence T cells show activation markers despite therapy.

2. Serum levels of SFas are elevated in patients with ANCA positive vasculitis at onset of the disease and the levels remain elevated despite remission suggesting a disturbance in mechanisms regulating apoptosis. These disturbances may result in the failure to terminate immune responses and control autoreactive lymphocytes.

3. CD26 is an inhibitor of cell surface expression of endogenous TSP-1 in T cells. Chemokines stimulate cell polarity and migration through abrogation of the CD26-dependent inhibition of TSP-1 expression and TSP-1 induces CD91 expression suggesting that T lymphocyte motility is regulated by a cascade of interacting cell surface molecules.

4. The CD26-controlled chemokine-activated TSP-1- CD91 cascade seems to be disturbed in patients with ANCA-positive vasculitis and these patients also showed increased or depressed chemokine levels in the circulation.

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