Systemic immune markers characterizing early stages of rheumatoid arthritis
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Document Version
Publisher's PDF, also known as Version of record

Publication date:
2016

Link to publication in University of Groningen/UMCG research database

Citation for published version (APA):

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Chapter 8

Chronic autoimmune mediated inflammation, a senescent immune response to injury

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The increasing prevalence of chronic autoimmune-mediated inflammatory diseases (AIMID) in ageing western societies implies a major challenge for the drug development industry. The current high medical need for more effective treatments is at least in part caused by our limited understanding of the mechanisms that drive chronic inflammation. Here we postulate a role for immunosenescence in the progression of acute to chronic inflammation via a dysregulated response to primary injury at the level of the damaged target organ. A corollary to this notion is that treatment of acute versus chronic phases of disease may require differential targeting strategies.
The immune system: for better or for worse

The primary function of the immune system is to protect the body against the detrimental effects of infection with viruses, bacteria or parasites, while at the same time damage to the infected tissues should be minimized. Although many threats come from the environment, also a healthy body carries many potential pathogens that need to be controlled, such as the bacteria that layer body surfaces (gut or skin) and the viruses in blood, lymphoid organs and bodily tissues. For its protective task the immune system is equipped with defense functions, which are in part already fully operational at birth (innate immunity) or which mature after birth in the daily engagement with environmental cues (adaptive immunity). While most people enjoy the benefits of immune protection for securing a healthy life, a substantial and steadily increasing proportion of the population, in 2000 ± 25% of the population in the USA; (http://mpkb.org/home/pathogenesis/epidemiology), experiences the hazardous consequences of unwanted and often detrimental immune activities. Examples of such conditions are allergy and autoimmunity, which are both driven by a dysregulated hyper reaction of the immune system. In the case of allergy the response is directed against environmental factors (e.g. pollen, food components, chemicals) and in the case of autoimmunity against components from body cells and tissues.

Autoimmune-mediated inflammatory disease (AIMID)

Inflammation results from the body response towards infection, irritation or tissue injury and is mediated by the immune system. Depending on severity, the clinical features of inflammation - pain, heat and swelling – can cause impairment of function. Inflammation is a complex biological process in organs and tissues aiming at the elimination of injurious factors and activation of the healing process. In a healthy individual, inflammation usually wanes when the insult has been eliminated and/or the injury has been healed. However, in certain clinical conditions inflammation does not wane but persists for prolonged periods of time. Chronic inflammation can occur for example when the immune reactions that drive the inflammation are directed against self-antigens present in or released from injured tissues.

The clinical course of AIMID is often characterized by an early phase dominated by inflammation with relatively more inflammation than tissue erosion, which can be treated with reasonable success using currently available immunotherapies, and a late phase where tissue degeneration is more pronounced than inflammation, for which an effective treatment is often lacking.

The lack of effective treatments for the chronic phase of AIMID is due to our limited knowledge of the mechanisms that underlie chronic inflammation and the lack of valid animal models (1). The poor predictive value of current AIMID animal models is a major hurdle in the translation of new therapeutic principles from the laboratory bench to the hospital bed (2).

Chronic inflammation

In most AIMID types the triggering event(s) is (are) not known. However, the subsequent exacerbations and remissions of clinical symptoms are believed to be mediated by the immune system. The exposure of
genetically predisposed individuals to (an) environmental trigger(s), infections in particular, has been proposed as a likely ethiogenic event, inducing the activation of autoreactive T and B cells present in the normal repertoire. The activation of autoimmune cells alone is usually not sufficient for the induction of overt clinical symptoms of autoimmune disease. Often additional pathogenic events within the target organ need to occur as well, including the activation of local APC and tissue injury, leading to the release of self-antigens and danger signals, named damage-associated molecular patterns (DAMPS), such as mitochondrial DNA (3), stress proteins (hsp60, hsp70) (4) or nuclear factors (e.g. HMGB1) (5).

As chronic AIMID are most prevalent in elderly people, it is thought that also age-associated changes in the immune system or the target tissues of the autoimmune attack enhance the risk of chronic inflammation, although the exact underlying mechanisms are poorly understood (1). The latter assumption warrants the question which age-associated changes of immune function enhance the risk to develop chronic AIMID.

**MS and its animal model EAE, examples of prototypical AIMID**

The difficulty to translate pathogenic and therapeutic concepts from the laboratory to the clinic can be illustrated by the situation in multiple sclerosis (MS). MS is a complex autoimmune-driven inflammatory disease affecting the human central nervous system (CNS), comprising the brain and spinal cord. The autoimmune pathogenesis of MS is modeled in experimental autoimmune encephalomyelitis (EAE). EAE can be induced in a variety of animal species (mice, rats, guinea pigs, primates) by active immunization with CNS antigens, mostly derived from the myelin sheaths that enwrap axons forming an isolation layer that facilitates fast pulse conduction (6). Although the EAE model has been instrumental for the development of several immunomodulatory/anti-inflammatory therapies (7), it has also been criticized as being an unreliable preclinical model (8,9).

Based on the response to treatment with immune modulating anti-inflammatory therapies, two phases can be distinguished in the pathogenesis of MS (6). Acute inflammation in the early disease phase responds well to some immunomodulating anti-inflammatory treatment, whereas inflammation in the late-stage chronic phase usually responds much poorer to these treatments. A representative example may be the beneficial effect of interferon-β on inflammation within the CNS white matter in relapsing-remitting MS and in EAE models, as detected on magnetic resonance images, whereas it has only a poor, if any, effect on clinical progression (10).

This discrepancy raises important questions:

1. Are early-acute and late-stage chronic disease driven by different pathogenic mechanisms?
2. Which immune alterations accompany or are at the basis of the transition from acute to chronic disease?
3. Which genetic and/or environmental risk factors steer the acute to chronic phase transition?
4. Is there experimental evidence for the existence of different immunopathogenic mechanisms in acute and chronic AIMID, from the EAE model for example?

**Pitfalls of experimental disease models**

While considering the relevance of animal models for the research of human AIMID the famous quote of the statistician George Box should be kept in mind: “*All models are wrong, but some are useful*” (11). Indeed, there is no animal model that recapitulates the complexity of human AIMID. However, this does not imply that all animal models are useless, as they can be of great help for the modeling of certain pathogenic mechanisms.

Despite the limitations, animal models have an important role in the preclinical research into disease mechanisms and the development of new therapies. Over the years, the inbred/SPF laboratory mouse has become the most frequently used animal model in preclinical AIMID research. However, it becomes increasingly clear that the immunological gap between a 10-12 weeks old SPF-bred mouse from a genetically homogeneous (inbred) strain and the complex patient population is more challenging than previously perceived and that this contributes to the frustrating situation that many new therapeutic entities fail to reproduce promising effects observed in a disease model when they are tested in patients.

Hence, the question arises what can be learned from models in species that are more closely related to humans. Again, we use MS and its animal model EAE as an example.

The choice for a suitable animal model should be guided by the risk factors that have a well-documented influence on MS:

1. **Genes**: All genetic association studies reveal that the strongest genetic influence on MS susceptibility is exerted by the major histocompatibility complex (MHC) (12). This polygenic and highly polymorphic genomic region encodes molecules involved in antigen presentation to CD8+ and CD4+ T cells (MHC class I and II region) as well as effector molecules and their receptors (class III region).

   While selecting an animal model for translational research into AIMID pathogenesis and therapy, close genetic resemblance with humans enhances the relevance of the model.

2. **Environment**: Environmental factors with a recognized influence on the initiation and progression of AIMID are infection and vitamin D (13). We will not discuss the mechanism of action and therapeutic perspectives of vitamin D here and like to refer to reviews elsewhere (14) (15). An important difference between humans and laboratory rodents is that the human immune system has been shaped by the day-to-day exposure to new and existing infections. Viruses causing lifelong opportunistic infections, such as herpes viruses (CMV, EBV), and the bacteria in our gut flora (microbiota) have a particularly important impact on the human immune system.

In both respects, outbred colonies of conventionally housed non-human primates provide useful models for narrowing the gap between AIMID models in inbred/SPF rats and mice and the human population.
3. **Age**: The human immune system undergoes many changes associated with ageing of the body, which seem to be expressed more in the adaptive than the innate arm of the immune system. Documented changes include thymic involution, conversion of the CD4/CD8 ratio, decrease of the proportion of naïve T cells and progressive clonal expansion of terminally differentiated T cells lacking surface expression of CD28 (CD28null) (16). The functional consequences of this immunosenescence process are reduced responses to vaccination, increased immune reactivity against autoantigens and an increased systemic inflammatory state (inflammaging). Although the exact mechanisms underlying these changes of immune function are incompletely understood, the chronic inflammatory state has been associated with clonally expanded, pro-inflammatory CD28null T cells (16). Here we postulate that the CD28null T cell subset has a direct pathogenic role in chronic inflammation and may thus be considered as a potential target of therapy.

Many of the age-related alterations observed in the non-human primate immune system resemble those in humans, including the oligoclonal expansion of CD28null T cells that mediate inflammaging at the expense of naïve T cells that can respond to new antigenic challenge (vaccination). Of note, CD28 loss is not observed in murine systems, thereby adding to the notion that mouse models do not fully capture immunosenescence features as observed in man (1). These arguments plead for the non-human primate as inevitable preclinical model in drug development for AIMID. It should be noted that this is already common practice in transplantation immunology where non-human primates were proven to be better predictors for clinical success of new immunomodulatory treatments than rodents (17).

**EAE in nonhuman primates**

EAE has been induced in two macaque species, the rhesus (*Macaca mulatta*) and cynomolgus monkey (*Macaca fascicularis*) (for review: (18)). However, the ensuing disease is usually acute and seriously destructive, showing distant resemblance with the chronic progressive disease course in MS. The more recently developed EAE model in common marmosets (*Callithrix jacchus*) is much less severe and more heterogeneous in its clinical and pathological presentation than the rhesus monkey model, comprising cases with acute short-lasting disease and cases with chronic long-lasting disease (6).

Of the many CNS myelin components that can be used for EAE induction, the quantitatively minor, albeit specific, constituent myelin/oligodendrocyte glycoprotein (MOG) was identified as the most important autoantigen for induction of chronic disease in marmosets. This is best illustrated by the observation that marmosets immunized with MOG-deficient mouse myelin fail to develop chronic EAE, whereas their fraternal twin siblings do develop chronic disease (19). As an unglycosylated recombinant protein expressed in E. coli and formulated with the strong bacterial adjuvant CFA, MOG induces clinically evident EAE in almost 100% of marmosets from our outbred colony, but the disease course varies (20). Based on immune profiling data and the response to immunotherapy, we could conclude that acute
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Inflammation in the early disease phase and chronic inflammation in the late phase are driven by different immunopathogenic mechanisms (see Figure) (6).

**Figure 1. Two pathways leading to EAE in marmosets.**

Immunoization of marmosets with rhMOG induces Th1 cells and the production of autoantibodies by B cells. The Th1 induce CNS inflammation, whereas binding of the autoantibodies to myelin sheaths induces demyelination via macrophage and complement dependent cytotoxicity. The initial autoimmune attack via this **classical pathway** elicits the release of autoantigens, which drain to cervical and lumbar lymph nodes, where (effector memory, EM) T cells are activated. These are characterized by high IL-17A production and specific cytotoxicity. It has not been elucidated whether these two activities are mediated by two different T cell types (Th17 and CTL) or that one T cell type (IL-17^+^CTL) mediates both activities. The lack of CD28 expression and crossreaction with an immunodominant antigen of cytomegalovirus (major capsid protein; UL86) hints at the possibility that the EM cells may originate from the anti-viral memory repertoire. The secondary autoimmune attack via this **non-classical autoimmune pathway** results in pathological characteristics of progressive MS, i.e. microglia activation and demyelination of cortical grey matter. B cells are involved in the activation of the T cells mediating this progression pathway. Abbreviations: CFA = complete Freund’s adjuvant; DC = dendritic cell; MΦ = macrophage or microglia cell.

**Early phase EAE:** The early EAE phase in marmosets is driven by a canonical autoimmune mechanism that is strongly reminiscent of the EAE models in mice and rats (6). The inoculation of rhMOG/CFA into the dorsal skin elicits a uniform immunological event in all monkeys, namely the activation of T helper 1 cells specific for the epitope MOG24-36 together with autoantibodies against conformational MOG epitopes. The uniformity of the EAE initiation was explained by the fact that the MHC class II restriction element is a monomorphic MHC class II allele (**Caja-DRB1*W1201**) (21,22), which is ubiquitously
expressed in common marmosets (23). The synergistic action of Th1 cells and autoantibodies induces besides inflammation (22) and demyelination (24) also reversible axonal injury mainly localized in the white matter (25). Disease development in this early phase can be stopped by treatment with anti-human IL-12p40 antibody (26) or anti-CD40 antibody (27). The pathogenic mechanisms and response to therapy are very similar to those expressed in corresponding mouse EAE models.

Late phase EAE: The late EAE phase is driven by an unconventional autoimmune mechanism that has not yet been found in SPF rodent models but, as discussed elsewhere (6), a similar mechanism may be operational in MS. The variable onset of clinically evident EAE was found associated with the reactivation of CD3+CD4+CD8+CD56+CD27+CD28- effector memory cytotoxic T cells specific for MOG34-56 (28). The signature cytokine of this subset is IL-17A (29), but neutralization of IL-17A with a human-anti-human IL-17A antibody exerted little clinical effect (30). The specificity of the cytotoxic cells was defined at peptide 40-48 and the MHC restriction at the non-classical MHC class Iβ allele Caja-E (31). In the Immuno Polymorphism (IPD)-MHC database (http://www.ebi.ac.uk/ipd/mhc/nhp/) only two Caja-E alleles have been published (Caja-E*0301 and –E*0302), which differ by a single nucleotide (triplet 138 ACG -> ACC). As the encoded amino acid is located outside the peptide-binding groove (position 107), the MHC class I molecules encoded by both alleles are likely functionally identical. The observation that the CD8+ T cells from monkeys sensitized against MOG34-56 cross react with peptide 981-1003 from the CMV major capsid protein (32) and that this response is MHC-E restricted point to a possible relation with a recently identified subset of HLA-E restricted NK-CTL in the human repertoire, which are engaged in the control of CMV infection (33,34). Based on this similarity we hypothesize that the CD3+CD4+CD8+CD56+CD27+CD28- effector memory T cells that have a core pathogenic role in the late EAE phase in marmosets and can be activated by immunization with MOG34-56 in IFA, originate from anti-CMV memory T cells present in the natural immune repertoire.

As discussed elsewhere (6), T cells are the key mediators in the EAE pathogenesis in marmosets but also B cells have a critical albeit different pathogenic contribution to early and late stage disease (see Figure). In the classical Th1-mediated pathway inducing early EAE the role of B cells is to produce autoantibodies that induce demyelination via cellular or complement–mediated cytotoxicity reactions (ADCC and CDC). In the non-classical CTL-mediated pathway inducing late stage EAE the main role of B cells is antigen presentation.

In summary, the similarities of the marmoset EAE model with MS include:
- the evidence for both an early acute and chronic phase of disease
- the almost immediate strong clinical effect of CD20+ B cells depletion (35,36),
- the involvement of CD3+CD28null T cells in chronic inflammation (37),
- the implication of CD3+CD4+CD56+ T cells in demyelination, by cytotoxic killing of oligodendrocytes (38,39).
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Chronic inflammation, a response-to-injury paradigm
Surgical removal of CNS draining cervical (brain) and lumbar (spinal cord) lymph nodes impairs the chronic relapsing disease course in a Biozzi ABH mouse EAE model (40). The similar localization of myelin-laden APC within these lymph nodes during the course of EAE in mice and marmosets (41) and of MS in patients (42) supports an important role of these lymph nodes in the disease pathogenesis. Similar to marmosets, the chronic relapsing EAE course in Biozzi ABH mice is driven by autoimmunity against MOG (43). Based on these observations we have postulated a response-to-injury model for EAE and MS, which implies that MS is caused by a predisposed dysregulated immune reaction against antigens released from a damaged organ (44). The assumptions underlying this postulate are:
1. that primary injury inflicted in an organ causes release of self-antigens that either passively drain to lymph nodes as free molecules or are actively transported by phagocytic cells.
2. that T cells present in these draining lymph nodes exert a dysregulated hyper reaction to the released self-antigens. Conceptually, the combination of genetic and environmental factors predisposes an individual to a dysregulated autoimmune hyper reaction.

The cause of the primary injury can be diverse, including i. acute inflammation, as modeled in EAE, ii) a vascular problem, iii) tissue degeneration, as in neurodegenerative diseases, iv) virus infection. Actually, MS seems to share many pathological similarities with the vascular disease atherosclerosis (45). We like to state here that the autoreactive CD28 negative NK-CTL that were identified as core pathogenic factor in the late phase of marmoset EAE are an example of T cells capable to exert a dysregulated hyper reaction eliciting chronic AIMID.

CD28null T cells and (chronic) inflammation
One of the prominent features of immune aging is the oligoclonal expansion of CD4+ and especially of CD8+ T cells that lack expression of the co-stimulatory molecule CD28 (16). The expansion of CD28null subsets seems to be oligoclonal and partly the consequence of replicative stress due to recurrent exacerbations of (latent) cytomegalovirus (CMV) infection (46). CD28null cells seem to have lost proliferative potential but demonstrate enhanced survival (47). CD28null cell function is characterized by proinflammatory cytokine production and expression of perforin and granzyme B suggesting their cytotoxicity. Moreover, CD28null cells are relatively insensitive to suppression by regulatory T-cells (46). Many individuals with elevated numbers of CD28null cells in their circulation suffer from autoimmune disease. However, these cells rarely respond to disease-specific autoantigens, but rather to antigens from CMV or EBV (46) or to stress proteins, such as heat-shock protein (hsp) 60 (48). The central question therefore arises whether CD28null cells may be generic drivers of chronic inflammation in AIMID.

Is there a mechanistic explanation for a role of CD28null cells in the dysregulated immune reaction to injury? The CD28null effector memory T cell population acquires expression of several NK receptors
(both activating and inhibitory receptors). This may be a loss of function compensatory mechanism (49) mediated by reduced DNA methyl transferase activity, allowing the expression of methylation sensitive genes (50). Indeed, the methylation status of T cell derived DNA was recently shown to be age-dependent (51). The most frequently expressed NK receptor on CD28null cells is KIR2DL4 (CD158d), an activating receptor despite the presence of an immunoreceptor tyrosine-based inhibition motif (ITIM) in its cytoplasmic tail (52). CD70 is another methylation sensitive gene that is expressed by CD28null T cells. CD70 expression may contribute to enhanced survival (53). Also, CD70 was found to lower T cell activation thresholds (54). Loss of CD28 associated with prominent expression of CD70 and de novo NK receptor expression infers that CD28null T cells are less dependent on cognate signaling (TCR/CD28) and thus sense their environment differently using receptor ligand (KIR-MHC class I) interactions characteristic of the innate immune system. Moreover, as CD28 loss is associated with increased production of pro-inflammatory cytokines and expression of cytotoxic effector molecules, CD28null T cells not only sense their environment differently but also are likely to respond differently and thus may be mediators of the dysregulated immune reaction to tissue injury.

In which AIMID has a possible pathogenic role of CD28null cells been documented?

Several chronic inflammatory diseases, including rheumatoid arthritis (RA), systemic lupus erythematosus, Wegener’s granulomatosis (GPA), atherosclerosis, inflammatory bowel disease and MS, are all characterized by expansions of CD28null T cells in the blood. Importantly, CD28null T cells have been detected at the site of pathology, suggesting their contribution to the disease process (MS and atherosclerosis). Here, we will briefly summarize the findings on CD28null T cells in RA, MS and in atherosclerosis.

RA: Weyand and Goronzy have reported on high relative percentages of CD4+CD28null (up to 30-40% of CD4+ T cells) in patients with RA (55). Interestingly, premature accumulation of CD28null cells was found associated with carriage of the RA-associated HLA-DR4 subtypes. On the basis of these findings a novel disease hypothesis for RA was proposed (56, 57). In subsequent studies, the expansion of CD28null cells in RA was confirmed in one third of patients and was linked to CMV seropositivity. Moreover, the expansion of CD28null cells was linked to the expression of the RA-associated HLA-DR4 subtypes in both RA and healthy controls (58). Also, according to a recent study anti-CMV seropositivity of RA patients, which is associated with increased frequencies of CD28null T cells and CMV-specific Th1 cells, was linked to a more severe disease course (59). Notably, CD4+CD28null cells were most frequently found in patients with extra articular disease manifestations (e.g. vascular pathology).

It was previously suggested that CD4+CD28null cells by virtue of CD161 expression home to the synovial tissue in RA (60). CD161+ cells were found in synovial tissue but CD28 expression was not assessed. Later, others failed to demonstrate the presence of CD28null at the site of pathology in RA (61). Indeed, our own observations, imply a role for CD4+CD161+ effector memory Th1 cells in RA synovitis.
MS: Expansions of pro-inflammatory CD4+CD28null T cells in the peripheral blood of MS patients are less frequent than in RA but have been reported by several groups (47,62). As in RA, a correlation with CMV seropositivity was established. Importantly, CD4+CD28null cells were detected in the cerebrospinal fluid and in the inflammatory lesions in the brain. Mechanistically, the fractalkine – CX3CR1 pathway was found involved in the migration to the target tissue (63).

Compared to RA, relatively little mechanistic information is available on the pathogenic contribution of CD28nullCD161+ T cells in MS. In a genome-wide association study CD161 emerged as a candidate susceptibility locus in MS (64). In MS, upregulation of CD161 expression on IL-17+CD8+ T-cells, has been reported. These cells were further characterized as CCR6+ EM cells (CD27−/−CD45RA−) with a pro-inflammatory profile and lack of perforin (37). The expression of CCR6 may enable these cells to immigrate non-inflamed CNS via a recently discovered route that circumvents the blood brain barrier i.e. via the choroid plexus where high expression of the CCR6 ligand CCL20 has been observed (65). CD4+ T-cells that express CD161 can differentiate into Th17 EM cells, a cell type with a presumed prominent pathogenic role in MS (66).

Atherosclerosis: Atherosclerotic vascular disease (atherosclerosis/ASVD) is a complex progressive inflammatory disease affecting the cardiovascular system. ASVD is an important co-morbid condition in patients with RA. The disease is pathologically characterized by inflammation and thickening of arterial walls. In the arterial walls both stable and unstable atherosclerotic plaques are found. Stable plaques, which usually cause limited or no clinical problems, mainly consist of extracellular matrix and smooth muscle cells. Unstable plaques also contain inflammatory cell infiltrates; these plaques can rupture and release thrombogenic material into the circulation causing the cardiovascular problems. Besides (lipid-laden) macrophages, T cells are consistently found in atherosclerotic lesions (67). In the early stages of atherosclerosis CD4 T cells (LDL specific) are held responsible for the initiation and progression of the disease, whereas in the advanced stage, a role for CD4+CD28null T cells in mediating atherosclerotic plaque instability was shown (68,69). CMV establishes persistent infection of arterial cell walls and CD4+ T cells specific for CMV contribute to atherosclerosis development (70). In certain clinical conditions, HIV-associated atherosclerosis for example, it could be shown that cardiovascular problems by CMV are mediated by CD4+CX3CR1+ T cells (71). In view of the prior discussion it is tempting to speculate, but unproven, that CMV sustains the activation of pro-atherosclerotic CD4+CD28null T cells within the vessel wall.

Conclusions and implications for therapy of AIMID

Chronic AIMID are often characterized by prolonged and persistent inflammation and by new connective tissue formation. It may be a continuation of an acute form or a prolonged low-grade form. In some AIMID, such as RA and MS, chronic disease is pathologically associated with the formation of ectopic lymphoid structures, respectively within arthritic synovium (72) and MS meninges (73). It is suspected
that these newly formed sites of lymphoid neogenesis support the persistent production of pathogenic factors, such as autoantibodies and pro-inflammatory cytokines (74). According to an intriguing, but disputed new concept, EBV-infected B cells play a central role in the organization of these structures (75,76).

In this viewpoint we propose a mechanistic concept of chronic inflammation in AIMID. For this discussion we have used literature data, our own experimental data from the prototypical AIMID animal model EAE and our own studies on immune ageing markers in RA. The assumption that clinically unrelated AIMID may share immune mechanisms that drive chronic inflammation, is based on the outcome of genome-wide association studies (77).

In summary, the postulated concept comprises the following elements:

1. Chronic inflammation is driven by a dysregulated T cell hyper-response against antigens released from a primary injury in a body organ. The pathogenic factor inflicting the primary lesion can come from within the organ, such as a degenerative or vascular problem, or from outside the organ, such as an acute inflammation caused by an autoimmune attack.

2. Depending on the nature of the pathogenic event that causes the primary lesion, the released antigens can be self-antigens chemically modified by post-translational processes or can be de novo synthesized, such as stress proteins. We assume that immune tolerance against such antigens is weak or non-existent.

3. The hyper-reacting T cells originate from a repertoire of effector memory T cells induced by antecedent viral infections. The genetic background of the individual (e.g. MHC class I and II polymorphisms) determines whether these anti-viral T cells crossreact with look-alike epitopes within self-antigens.

4. Immunoageing and the replicative stress by recurrent exacerbation of latent infections (e.g. CMV, EBV) are associated with oligoclonal expansion of CD28null KIR expressing T cells, which can be activated by antigens released from an injured organ. These cells are less sensitive to the normal immune regulatory mechanism, such as Treg cells and adrenal hormones (corticosteroids). The paradox that the expanding CD28null T cells are mostly CD8+, but that CD8+ T cells do not readily respond to soluble self antigens has been addressed in the marmoset EAE model. Accumulating evidence suggests that EBV-infected CD20+ B cells contribute to late stage EAE by presentation to the cytotoxic T cells of MOG34-56 via non-classical MHC class I molecules from the HLA-E lineage (unpublished own observations).

What are the implications of the pathogenic concept discussed in this publication for therapy development? The concept postulates that inflammation in AMID is driven by the reactivation of pre-existing effector memory T cells present in the normal immune repertoire. The fact that these memory cells are already committed to their lineage may explain the poor translation of immunotherapies
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Intervening with the activation of naïve T cells or their tolerization from AIMID animal models to the corresponding human disease. The concept also proposes that age-associated changes in the immune system, in particular the expanding repertoire of CD28null T pro-inflammatory cells, may explain why therapies targeting mechanisms of acute inflammation in MS – such as corticosteroids, β-interferons or glatiramer acetate - loose efficacy with progression of the disease. Notably, (repeated) corticosteroid treatment /use may even enhance immunosenescence through further (steroid-induced) thymus involution and thus may inadvertently contribute to the accumulation of CD28null T cells in chronic diseases.

Data obtained from the marmoset EAE model demonstrate a similar central pathogenic role for B cells as in MS (78). The underlying mechanism is that the core pathogenic NK-CTL need antigen presentation by B cells for their activation (79). Intriguingly, B cells infected with EBV are particularly equipped for this task (own unpublished observation). These findings may not only give a mechanistic explanation for the clinical efficacy of anti-CD20 antibodies in MS and RA and for the association of these AIMID with EBV, but also warrant the search for treatments that specifically target the EBV-infected B cell.

**Key messages:**
- We postulate a generic paradigm for AIMID
- Acute autoimmune inflammation causes injury in an organ
- Chronic inflammation is a dysregulated T cell reaction against antigens released from injury
- T cells lacking CD28 (CD28null) have a central role in the response to injury
- CD28null effector memory T cells are a hallmark of immune ageing

**Acknowledgements:**

We like to thank Mr. Henk van Westbroek (BPRC) for preparing the artwork.

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