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Review

New insights into the pathogenesis of giant cell arteritis[☆]



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ABSTRACT

Giant cell arteritis (GCA) is an inflammatory chronic disease occurring exclusively in elderly individuals. Until recently, the disease has been considered a unique disease resulting from the interaction in the walls of susceptible arteries, between an unknown infectious agents with local dendritic cells (DCs), activated CD4 T cells and effector macrophages. Recent evidence has shown that this view was too simplistic and has clarified many of the pathogenetic aspects of the disease. Many genetic studies recently published have identified different new genes, including cytokines, adhesion molecules and regulators of innate immunity, as crucial players in the development and progression of GCA. Recent evidence suggests that there is heterogeneity of histological lesions in GCA, that are correlated with different immunological Th9 and Th17 signature. The recent demonstration that Varicella-zoster virus (VZV) antigen is present in the 64% of GCA-negative TAs and in the 73% of GCA-positive TAs could represent an important point of arrival in the search for a causative agent in the pathogenesis of a metameric disease such as GCA. In this context, cytokines such as IL-32 and IL-33 that act as a danger signal following tissue damage and infection are over-expressed in GCA arteries. Artery tertiary lymphoid organs, present in up to 50% of GCA-positive arteries, could represent the sites where primary immune responses and T- and B-cell autoimmune responses against viral antigens are organized. The recently demonstrated disturbed distribution of B cells in GCA could be also relevant in the pathogenesis of the disease, possibly contributing to the enhanced IL-6 response. Altogether, these evidences may clarify many pathogenetic aspect of the disease, also suggesting complexity greater than first imagined.

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1. Introduction

Giant cell arteritis (GCA) is an inflammatory chronic disease occurring exclusively in elderly individuals [1]. GCA is clinically characterized by cranial symptoms such as headache and scalp tenderness [2]. In many patients a systemic inflammatory response may also occur with fever, weight loss and fatigue [2]. To date no specific circulating biomarkers have been identified and the gold standard for the diagnosis of GCA remains the biopsy of temporal artery (TAB) [3–5]. Histologically, arteries show inflammation and necrosis of the arterial media wall, infiltrating CD4⁺ T lymphocytes, macrophages multinucleated giant cells and/or epithelioid macrophages with the production of various cytokines and arterial remodelling [6,7]. The aetiology and the mechanisms underlying the pathogenesis of GCA are still now unknown. The evidence that GCA occurs almost exclusively in individuals older than 50 years of age and that the incidence increase progressively after 50 years of age indicates that age-related immune alterations, in genetically predisposed subjects, are essential in the development of the disease [1,8]. Historically, GCA has been considered a unique disease resulting from the interaction, in the walls of susceptible arteries, between unknown antigens with local dendritic cells (DCs), activated CD4⁺ T cells and effector macrophages [9–11]. Recent evidences have shown that this view is too simplistic and have clarified some of the pathogenetic aspects of the disease. In this review, we summarize the recent findings regarding the pathogenesis of GCA.

1.1. Histologic heterogeneity in GCA arteries

The classic histologic picture of GCA is characterized by panarteritis with a predominantly lymphomononuclear inflammatory cell infiltrate, with or without giant cells [5]. Recently, however, it has been demonstrated that together with transmural involvement, the inflammation may be restricted to the vasa vasorum (vasa vasorum vasculitis, VVV), to the periadventitial small vessels (small vessel vasculitis, SVV), or both [12]. To clarify the clinical significance of SVV and/or VVV, Restuccia et al. compared patients with these lesions with a group of randomly selected patients with classic GCA [13]. Taken together, patients with SVV and/or VVV had less frequent cranial manifestations and lower levels of markers of inflammation at diagnosis compared to the control group with classic GCA, whereas the frequency of cranial ischaemic events was similar between the 2 groups. The Authors found that the clinical features of patients with isolated VVV were similar to those of the patients with classic GCA. Unlike isolated VVV, SVV seems to identify a GCA subset with distinct clinical features. In particular, patients with SVV were characterized by less frequent cranial manifestations and systemic symptoms and signs, by lower levels of acute-phase reactants at diagnosis, and by more frequent peripheral synovitis [13]. It is unclear whether the involvement of the peri-adventitial small vessels or vasa vasorum could represent an early stage in the development of classic transmural inflammatory infiltration. However, the evidence that the time from the onset of symptoms to diagnosis was similar in patients with SVV, isolated VVV, and classic transmural GCA and that the type of inflammatory involvement was the same throughout the excised temporal artery segments may suggest that SVV and VVV are not earlier stages of an inflammatory process culminating in transmural vessel inflammation.

1.2. Role of infectious agents

An infectious cause has been longer suspected for both GCA [9]. Cyclic fluctuation of GCA, with peak incidence rates every five to seven years, and seasonal fluctuation in the incidence of biopsy-positive GCA, with peaks in late winter and autumn have been demonstrated suggesting that solar exposition [14] or seasonal infections may influence the GCA pathogenesis [15]. Different infectious agents such as Chlamydia pneumoniae [16], Parvovirus B19 [11] and Epstein Barr

Virus [17] have been suggested to be involved in the pathogenesis of GCA. Rigorous studies, however, failed in demonstrating a unique or dominant role of these infectious agents. Recently, an association of GCA with varicella zoster virus (VZV) has been documented [18]. VZV is a human neurotropic alpha herpes virus that is able to replicate in arteries causing disease [19]. A productive VZV infection in cerebral arteries after either primary infection or reactivation of VZV has been described. VZV vasculopathy causes ischaemic infarction of the brain and spinal cord, as well as aneurysm, subarachnoid and cerebral haemorrhage, and carotid dissection [19]. In GCA patients VZV antigen has been found in the 64% of GCA-negative temporal arteries (TAs) and in the 73% of GCA-positive TAs, compared with 22% of normal TAs with a relative risk [RR] of 2.86 [18]. VZV antigen was more likely to be present in the adventitia of both GCA-negative TAs (RR = 2.43) and GCA-positive TAs (RR = 2.03). VZV antigen was frequently found in perineurial cells expressing claudin-1 around nerve bundles. VZV has been recently proved to downregulate programmed death ligand 1 (PD-L1) and MHC (Major Histocompatibility Complex)-1 expression in infected human brain vascular adventitial fibroblasts and perineurial cells, thus inducing persistent inflammation leading to pathological vascular remodelling [20]. A single group, however, has defended this hypothesis, and some investigators have questioned the specificity of the antibodies used in the initial studies [21]. Moreover, other investigators have not been able to confirm this association and this hypothesis has not been widely validated. More recently, the association between prior infections, in particular herpes zoster, and incident GCA has been studied in a population-based cohort. In this study, the Authors found that antecedent infections were only moderately associated with incident GCA, therefore suggesting that infectious agents are probably a minor determinant of overall risk of GCA [22].

1.3. Immunogenetics

Familial aggregation with sharing of HLA (histocompatibility leucocyte antigen) alleles has been described in GCA pointing to an important genetic component in the susceptibility for this vasculitis [23,24]. GCA has been associated with MHC class II in many independent studies and particularly with of HLA-DRB1*04 alleles even if not all studies have demonstrated a significant association. In a study performed on GCA patients from Rochester, Minnesota, USA, it has been proposed a DRYF motif at positions 28–31 in the second hypervariable region (HVR2) of MHC class II as a risk factor for GCA development [25]. However, this result has not been confirmed in a recent meta-analysis [26] and in a multicenter immunochip study [27]. Further studies have also demonstrated an association between HLA-DRB1*04 and visual loss [28] and glucocorticoid resistance [29]. A recent meta-analysis of published data on HLA-DRB1 associations of GCA, confirmed the strong association of GCA with HLA-DRB1*04 allele carriage, also identifying a possible protective effects of other alleles such as HLA-DRB1*01 and HLA-DRB1*15 [30]. A large-scale genetic analysis reveals a strong contribution of the HLA class II region to GCA susceptibility. In this study, DRβ1 13 and HLA-DQα1 47, 56, and 76 were identified as relevant positions for GCA susceptibility [30]. Differently from MHC class II alleles, only a weak association has been demonstrated with MHC class I alleles such as HLA-A*31, HLA-B*8, HLA-B*15, HLA-Cw3, HLA-Cw6 and MHC class I polypeptide-related sequence A (MICA) [24]. Outside the HLA region, the most significant loci included the protein tyrosine phosphatase non-receptor type 22 (PTPN22) [31] and the leucine rich repeat containing 32 (LRR32) [31]. More recently, Carmona et al. published a GWAS demonstrating that PLG and P4HA2 are risk genes at the genome-wide level of significance. PLG and P4HA2 are involved in vascular remodelling and angiogenesis, processes involved in the pathogenic mechanisms underlying GCA [32]. Coit P et al. recently performed a DNA-methylation study in temporal arteries from patients with GCA and controls [33]. In this study, DNA methylation data suggest a role for increased activity of the calcineurin/nuclear factor of activated T

cells (NFAT) signalling pathway in GCA. Further, other genes such as TNF, LTA, LTB, CCR7, RUNX3, CD6, CD40LG, IL2, IL6, NLRP1, IL1B, IL18, IL21, IL23R and IFNG were hypomethylated in the cellular milieu of GCA arteries [33]. Other susceptibility loci for GCA outside the HLA region have been identified by candidate gene studies including i) genes encoding cytokines or their receptors (tumor necrosis factor (TNF) α , interferon (IFN) γ , interleukin (IL) 10, IL-4, IL-6, IL-17, IL-18, IL-21, IL-33, monocyte chemoattractant protein-1 (MCP-1), chemokine (C-C motif) ligand 5 (CCL5), ii) genes involved in the Th (T helper) 1, Th2, Th17 and T regulatory cells (Treg) functions, iii) genes encoding molecules involved in the endothelial function (intercellular adhesion molecule 1 (ICAM-1), vascular endothelial growth factor (VEGF), nitric oxide synthases (NOSs), matrix metalloproteinase 9 (MMP9) and iv) genes of the innate immune responses (Toll-like receptor (TLR) 4, Fc γ R (Fc fragment of IgG receptor γ), myeloperoxidase (MPO), PTPN22, NLR family pyrin domain containing 1 (NLRP1) [34–54]. Despite these evidences, the reduced sample size and lack of replication in independent cohorts made the identification of homogeneous genetic association signals in GCA difficult.

1.4. Artery tertiary lymphoid organs in GCA arteries

Artery wall is an immune-privileged site characterized by the inefficient clearance of virus and the failure of T cells and macrophages to enter the virus-infected elastic media layer. This immune-privilege is lost with age as demonstrated by the presence, in elderly people and in advanced atherosclerotic lesions, of adventitial lymphoid infiltrates

ranging from tight clusters of B cells and T cells to highly organized structures sometimes comprising functional germinal centres, the so-called artery tertiary lymphoid organs, ATLOs [55]. Tertiary lymphoid organs (TLOs) develop at sites of inflammation where they influence the course of infection and autoimmune diseases [56]. Recently, distinct ATLOs structures, including placed B cell aggregates with a follicular dendritic cell (FDC) network, loosely surrounded by T cells, and the extensive formation of high endothelial venules, have been demonstrated in GCA patients [57] (Fig. 1). These GCA lymphoid aggregates, differently from “classic” ATLOs, were mainly observed in the media layer of inflamed arteries, being not associated with the age of patients, and/or with the occurrence of atherosclerotic lesions, and were independent by the degree of arterial inflammation [57]. Chemokine (C-X-C motif) ligand 13 (CXCL13) and chemokine (C-C motif) ligand 21 (CCL21) have been demonstrated to be instrumental in maintaining FDC networks and IL-7/IL-17 axis has been also demonstrated to be required for the organization of ectopic lymphoid structures [58]. In particular, CXCL13-attracted B cells, that home to the follicles, are the source of lymphotoxin (LT)- α 1 β 2 [59], which is critical for the generation and maintenance of established follicles. Analysis of tissue expression of cytokines, chemokines and their receptors in ATLOs positive GCA arteries, demonstrated a clear increased expression of IL-17 and IL-7, correlated with that of CXCL13, and of their receptor IL-7R and CXCR5 [57]. BAFF (B cell activating factor), APRIL (a proliferation-inducing ligand) and LT- β also involved in TLOs formation were significantly up-regulated in GCA arteries [57]. Interestingly, primary cultures of myointimal cells obtained from temporal arteries constitutively express large amount of

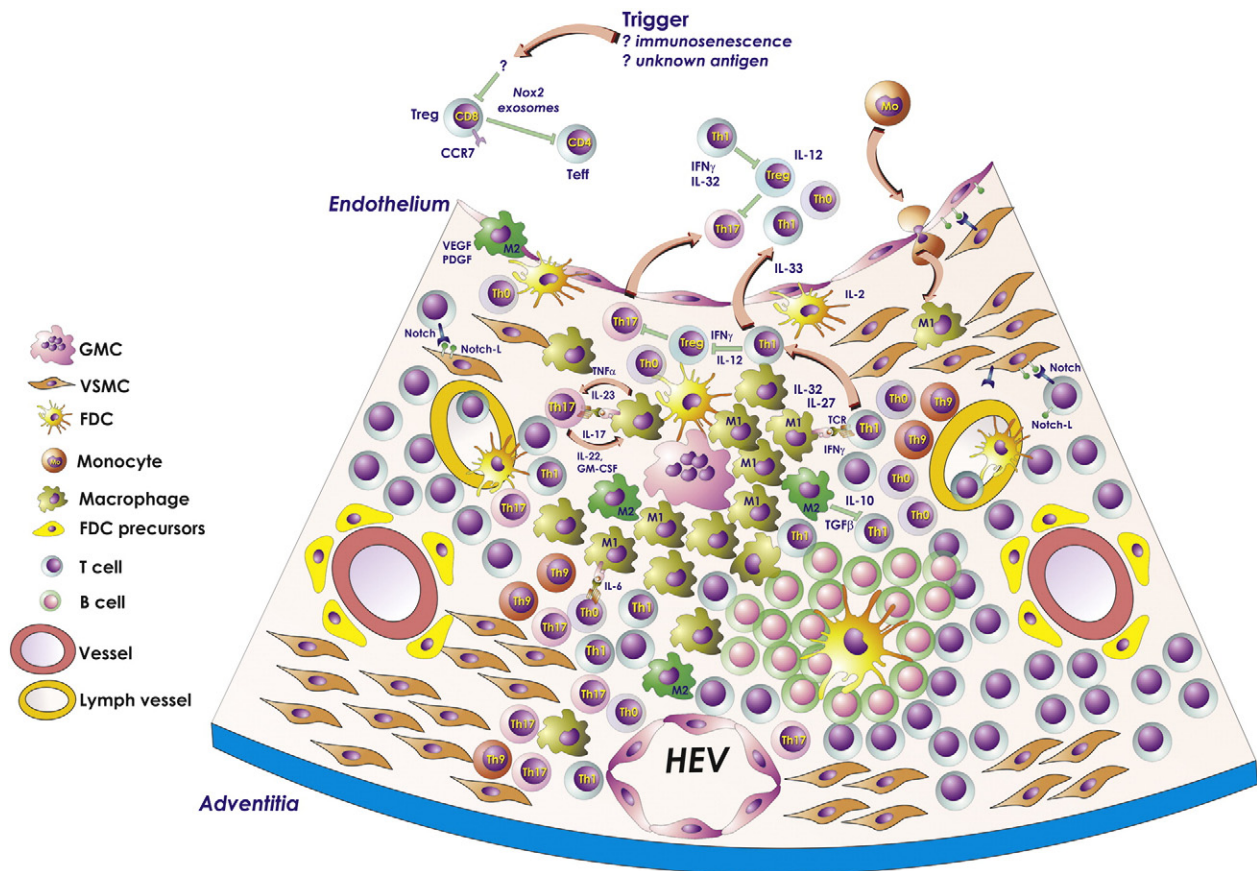


Fig. 1. The pathogenesis of GCA seems to be the result of an exaggerated immune response to an unknown antigen in susceptible individuals, usually older patients. Recent evidences suggest that this unknown antigen should be Varicella zoster virus. Immunosenescence seems to play an important role in the onset of illness possibly failing to generate immunosuppressive CD8⁺CCR7⁺ Treg cells. The artery immune-privilege is lost with age and is driven by activated dendritic cells, M1 and M2 polarized macrophages and CD4 T cells (Th1, Th9 and Th17 cells). Adventitial lymphoid infiltrates ranging from tight clusters of B cells and T cells to highly organized structures sometimes comprising functional germinal centres, the so called artery tertiary lymphoid organs, also participate in the disease pathogenesis possibly representing the immune sites where immune responses toward unknown arterial wall-derived antigens is organized. T cells, vascular smooth muscle cells (VSMC) and endothelial cells may interact by the interaction between NOTCH receptors and ligands regulating VSMC differentiation and plasticity, mediating VSMC–endothelial cell communication and promoting angiogenesis.

CXCL13, BAFF, APRIL and CCL21. TLR agonists and cytokines differentially regulated the expression of these chemokines in myointimal cells. TLR3 and TLR4 stimulation in fact induced in such primary cultures a strong and rapid up-regulation of both BAFF, CXCL13 but not of CCL21 and APRIL [57]. Conversely, stimulation with different pro-inflammatory cytokines involved in the pathogenesis of GCA, such as IL-1 β , IL-6, IL-17 and IFN- γ , differentially resulted in the significant up-regulation of BAFF, CXCL13, CCL21 and in a less manner of APRIL [57]. TLR3 and TLR4 are members of the TLR family which plays a fundamental role in the recognition of pathogen-associated molecular patterns (PAMPs) expressed on infectious agents, and mediate the production of cytokines necessary for the development of innate immunity [59]. Vascular myointimal cells have been demonstrated to express TLR3 and TLR4 and their stimulation, potentially triggered by infectious agents, might mediate the early release of CXCL13 and BAFF that seems to be essential in the lymphoid structures initiation by attracting lymphoid tissue inducer (LTi) cells and inducing their initial clustering [57]. The presence of ATLOs formation in GCA arteries suggests a role of these structures in disrupting the immune privilege of normal human arteries, possibly representing the immune sites where immune responses toward unknown arterial wall-derived antigens is organized (Fig. 1).

1.5. Dendritic cells activation in GCA

Experimental and clinical evidences highlighted in the recent past the primary role of cell-mediated processes in the pathogenesis of GCA and PMR. DCs, lymphocytes and macrophages are the predominant cellular components of the inflammatory infiltrate in affected vessels and also frequently contain multinucleated giant cells [10]. It has been historically proposed that the initiating immunological event is the activation of adventitial dendritic cells through the activation of TLRs [60]. Activation of diverse TLRs induces distinct cytokine/chemokine profiles that might be responsible for the loss of arterial immune-privilege [60]. Experiments performed in a mice model in which temporal arteries were subcutaneously engrafted into SCID mice have shown that allogeneic T cells do not recognize engrafted human arteries unless the immune privilege of the arterial wall has been broken by the activation of TLR4 with lipopolysaccharides [61]. Patients with GCA have altered TLR functions as demonstrated by the decrease TLR7 response during the acute phase of the disease [60] and the increased expression of TLR2 and TLR4 on arterial DCs [62]. TLR4-mediated DCs stimulation markedly enhances production of the chemokine CCL20, inducing the recruitment of CCL20-responsive CCR6⁺ T cells that dominate the vasculitic infiltrates in patients with panarteritic GCA (Fig. 1). Once activated, DC also release cytokines such as IL-6, IL-18, IL-23, IL-32 and IL-33 that activate pathogenic T lymphocytes in the wall of inflamed arteries [63]. The production of pro-inflammatory cytokines such as IL-6, IL-32 and IL-33 appears to closely correlate with the expression and severity of systemic symptoms [63] (Fig. 1). IL-6 is a pleiotropic cytokine that is associated with the production of acute phase proteins in hepatocytes, immunoglobulin induction in B-lymphocytes, and Th17 differentiation in T cells. IL-6 also is essential in orchestrating the pattern of immune reactions by driving the differentiation of naive T cells into Th17 cells. IL-6 is elevated in the serum of patients with GCA and over-expressed in the GCA temporal artery wall especially in those with systemic inflammatory response markers (such as weight loss, fever, haemoglobin <11.0 g/dL and erythrocyte sedimentation rate (ESR) > 85 mm). Interestingly, GCA patients with higher IL-6 levels experience more relapses and require higher doses of GC during follow-up. It has been recently demonstrated that the expression of IL-6 close depends on two different cytokines of the innate immunity, IL-32 and IL-33 signalling [64,65]. IL-32 is a recently described Th1-related pro-inflammatory cytokine with important functions in both innate and immune responses [66]. IL-32 expression is induced by Th1 cytokines, such as IL-1 β , TNF α , and IFN γ [66]. Human endothelial cells also constitutively produce IL-32, indicating IL-32 as a critical regulator of endothelial function through

modulation of coagulation, endothelial cell activation, and atherosclerosis [67]. IL-32 expression is markedly up-regulated in the inflamed arteries of patients with GCA [68] and it is accompanied by a strong overexpression of IL-27p28. IL-27p28 is a potent and earlier inducer of Th1 polarization [69] also inducing of IL-6 in rheumatoid arthritis (RA) fibroblast-like synoviocytes [70]. IL-6 expression by human arterial endothelial cells has been demonstrated to be also regulated by IL-33, a member of the IL-1 family, that after binding to its receptor ST2 (suppression of tumorigenicity 2), activates mast cells, Th2 lymphocytes and M2 macrophages and endothelial cells promoting angiogenesis and vascular permeability *in vitro* and *in vivo* [71]. An increased expression of IL-33 and its receptor ST2 has been found in GCA arteries mainly in endothelial cells of newly formed vessels [72]. The demonstration of IL-33 and ST2 intense endothelial positivity together with the positive correlation observed between IL-33 and the numbers of neovessels suggests a role of IL-33 in the pathogenesis of angiogenesis-dependent inflammation in GCA [72]. Interestingly, IL-33 expression was also correlated with the numbers of inflammatory parameters and reduced in steroid-treated GCA arteries [72]. These findings might indicate that IL-6-modulating cytokines could synergically participate in a positive-feedback mechanism, leading to the induction and perpetuation of IL-6 mediated arterial inflammatory immune responses.

1.6. Effector T cells in GCA

The evidence that identical CD4⁺ T cell clones are present in multiple GCA vasculitic sites, suggest a T cell response to a specific antigen [73]. CD4⁺ T cells play central roles in the function of the immune system by helping B cells, enhancing CD8⁺ T cells responses and regulating macrophage function in order to prevent autoimmunity and to modulate the intensity and the persistence of immune responses against pathogenic micro-organisms [74]. These pleiotropic functions are achieved through the differentiation of naive CD4⁺ T cells in effector and/or memory cells of specialized phenotypes such as Th1, Th2, Th9 and Th17 cells [74]. In particular, compared with control subjects, patients with GCA show a massive artery infiltration by IFN- γ -secreting Th1 [75,76], IL-9-secreting Th9 [77] and IL-17-secreting Th17 lymphocytes [75–77]. IFN- γ levels in the GCA arteries are correlated with neo-angiogenesis and the outgrowth of the hyperplastic intima [78]. IL-17 receptor is expressed on vascular smooth muscle cells (VSMC), fibroblasts, and endothelial cells that are affected by IL-17 stimulation and actively participate in vessel wall remodelling. The receptor for IL-9 activates endothelial cells, induces the expression of mast cell proteases and activates neutrophils [77]. Recent data suggest that changes in the composition of the T-cell infiltrate allow the distinction of the arteritis into early and late GCA [75,76]. Th1 cells and Th17 cells seem in fact to predominate in early phases of GCA [76]. Interestingly, a stronger IL-17A expression in GCA patients has been demonstrated to be associated with less relapses, also requiring significantly shorter treatment periods [79]. Differently from early GCA, chronic diseases seem to be characterized by the presence of glucocorticoid-resistant Th1 cells. A recent study evaluated the tissue distribution of Th1, Th9 and Th17 cells in GCA patients with different histological subsets, demonstrating a different representation of effector T cells [77] (Fig. 2). In particular, IL-17 overexpression seems to predominate in arteries with transmural inflammation and VVV. Differently from IL-17/Th17 cells, IL-9 overexpression and Th9 polarization predominate in arteries with transmural inflammation and SVV and its expression, together with interferon-gamma-producing Th1 responses, persist in treated patients [77] (Fig. 2). The role of T cells in GCA is confirmed by the recent study from [80] demonstrating a breakdown of the tissue-protective Programmed death1/Programmed death-ligand 1 (PD1/PD-L1) checkpoint. Engagement of PD-L1 with its receptor PD-1 on T cells inhibits TCR-mediated activation of IL-2 production and T cell proliferation [81]. Transcriptome analysis of GCA-affected temporal arteries revealed low expression of PD-L1 and concurrent enrichment of PD-1 receptor. DC cells from GCA

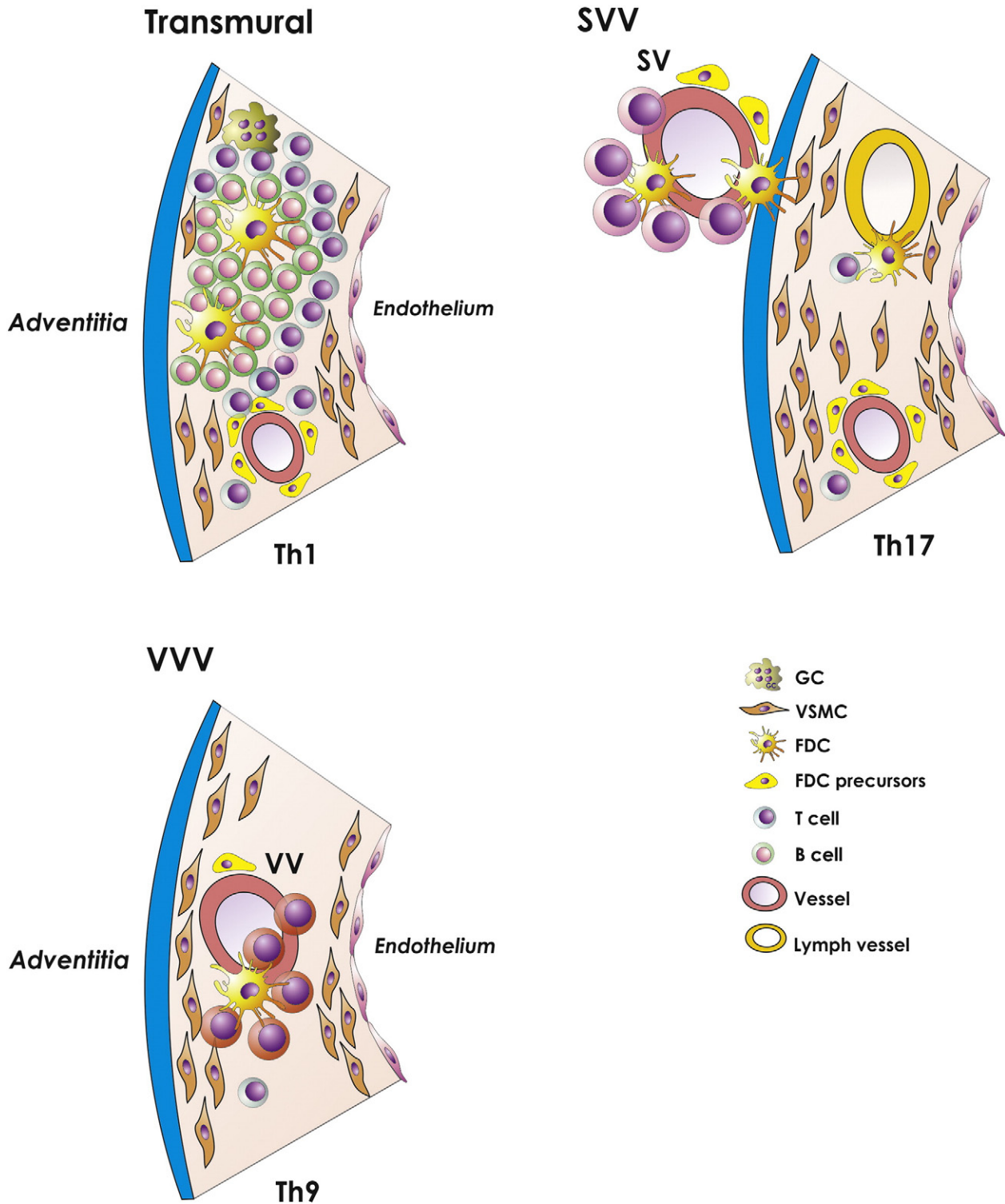


Fig. 2. Different subsets of CD4⁺ T effector cells are involved in the pathogenesis of different histologic subsets of GCA. Transmural inflammation seems to be essentially driven by a Th1 response. Small vessel vasculitis (SVV) is dominated by a Th1/Th17 response. Vasa vasorum vasculitis seems to be driven by a Th9 response.

patients were PD-L1^{hi}, whereas the majority of vasculitic T cells expressed PD-1, suggesting the lack of the immunoprotective PD-1/PD-L1 immune checkpoint. In human artery-SCID chimeras, PD-1 blockade exacerbated vascular inflammation, enriched for PD-1⁺ effector T cells, and amplified tissue production of IFN- γ , IL-17, and IL-21. Arteries infiltrated by PD-1⁺ effector T cells developed microvascular neoangiogenesis as well as hyperplasia of the intimal layer. A dysfunctional Tregs response has been also implicated in GCA pathogenesis

based on the demonstration of a reduced Treg cells frequency in the peripheral blood of GCA patients and the absence of any modulation of Treg cell frequency by steroid treatment [79]. Interestingly, the expression of Foxp3, the master regulator of Tregs is increased in GCA arteries [79,82]. Since that Treg cells display a remarkable functional plasticity, it might be possible that artery Tregs may not be suppressive actually producing Th1 or Th17 cytokines. In addition to CD4⁺ Tregs, CD8⁺ suppressor T cells are emerging as an important subset of regulatory T cells [83].

Diverse populations of CD8⁺ T cells with suppressive activities have been described [83]. Among them, a small population of CD8⁺CD25⁺-FOXP3⁺ T cells is found both in mice and humans [83]. CD8 lymphocytes have been demonstrated observed in early immunopathology studies performed in GCA arteries [84,85] and their expansion in peripheral blood was observed by Martínez-Taboada VM et al. [86]. A recent study by Samson et al. [87] demonstrated the implication and the prognostic value of CD8(+) T-cells in GCA. The Authors demonstrated that the percentages of circulating cytotoxic CD8 T lymphocytes (CTL, CD3(+)CD8(+)perforin(+)granzymeB(+)), Tc17 (CD3(+)CD8(+)IL-17(+)), CD63(+)CD8(+) T cells and levels of soluble granzymes A and B were higher in patients than in controls and reduced by steroids treatment. The intensity of the CD8 T-cell infiltrate in TAB was predictive of the severity of the disease. It has been recently demonstrated that older individuals fail to generate immunosuppressive CD8⁺CCR7⁺ Treg, a defect that is even more pronounced in the age-related vasculitic syndrome GCA [88]. In young, healthy individuals, CD8⁺CCR7⁺ Treg are localized in T cell zones of secondary lymphoid organs and act suppressing activation and expansion of CD4⁺ T cells. Wen Z et al. recently identified deficiency of NADPH oxidase 2 (NOX2) as the molecular underpinning of CD8⁺ Treg failure in the older individuals and in patients with GCA [88]. CD8⁺ Treg suppress CD4⁺ T cells activity by releasing exosomes that carry preassembled NOX2 membrane clusters (Fig. 1). Overexpression of NOX2 in aged CD8⁺ Tregs promptly restored suppressive function [88].

1.7. B cells responses

Beyond the role of T cells, B cells and humoral responses have been also showed in patients with GCA and PMR [89]. The demonstration of immune complexes in the serum of some patients with GCA and immunoglobulin deposits found along the internal elastic lamina of involved vessels in a minority of cases support a role of B cells in GCA [90]. A decreased numbers of circulating B cells is present in the peripheral blood of newly diagnosed patients with GCA or PMR [89]. B cell numbers recover rapidly in treated patients with GCA and PMR in remission and the B cell numbers are inversely correlated with ESR, C-reactive protein levels, and serum BAFF levels [89]. Although the role of B cells has been explicitly neglected by some investigators [10,86,91,92] their presence in GCA arterial lesions has been previously and repeatedly recognized by other investigators [84,85,93,94]. Further supporting the role of B cells in the pathogenesis of GCA is the demonstration that the presence of B cells, in a significant proportion of GCA arteries, is invariably associated with a defined T cell/B cell segregation surrounding a central area consisting of CD21⁺ cells (FDC) and with the significant over-expression of chemokines enhancing B-cell survival and expansion such as BAFF, APRIL and CXCL13 [57].

1.8. Neutrophils in GCA pathogenesis

Abundant neutrophils are present in the vasa vasorum and small vessels around temporal arteries in GCA [12,95] suggesting their involvement in GCA pathogenesis. Recently, the role of neutrophils in GCA pathogenesis has been studied by Nadkarni S et al. [96]. The authors hypothesized that persistent neutrophilia present at 24 weeks in GCA patients treated with steroids, might suggest the existence of a sub-clinical vascular inflammatory state that might explain disease re-emergence [96]. To test this hypothesis, the Authors analysed neutrophil phenotypes as early as 48 h after steroids and at 1, 4, and 24 weeks after therapy. GCA neutrophils display a classically activated CD16^{hi}AnxA1^{hi}CD62L^{lo}CD11b^{hi} phenotype at 48 h. This phenotype comes under rapid control within 1 week of treatment, despite stable neutrophilia, with a CD16^{hi}AnxA1^{hi}CD62L^{lo}CD11b^{lo} signature [96]. These neutrophils were hyporeactive, as confirmed by minimal interaction with an inflamed endothelial monolayer under flow conditions. In stark contrast, neutrophils at 24 weeks after glucocorticoids exhibited

a CD16^{hi}AnxA1^{hi}CD62L^{hi}CD11b^{hi} phenotype correlating with marked adhesion to endothelial monolayers. The Authors hypothesized that week 24 GCA neutrophils are unable to suppress T-cell responses, favouring loss of glucocorticoid control and, in time, re-emergence of vascular inflammation [96].

1.9. Mast cells in GCA

Mast cells (MCs) regulate different immunological responses causing allergy and autoimmunity and participating in tissue neovascularization through the secretion of a broad array of bioactive compounds [97]. In particular, it has been demonstrated that mast cells direct inhibit the functionality of Treg suppression thus actively participating in the establishment of Th17-mediated inflammatory responses. The presence of activated MCs in the neointima of GCA temporal arteries, in close spatial association with neovessels and T cells, has been recently demonstrated. The evidence that MCs express the receptors for IL-9 and IL-33, two cytokines over-expressed in GCA arteries may suggest a proangiogenic and immunoregulatory role of MCs in GCA [72,77]. Activated MCs may also participate in the remodelling of the affected arteries by regulating smooth muscle cells growth and death [98]. The exact role of MCs in GCA pathogenesis remains however still to be elucidated.

1.10. Role of endothelial cells in GCA

Endothelial cells are active player of vessel inflammation. Endothelial cells express molecules essential for cell-cell interactions, such as antigens from the MHC and the intercellular adhesion molecules. In GCA, endothelial cells are activated as demonstrated by the increased tissue endothelin-1 and endothelin-B receptor expression in temporal arteries. Endothelial cells of the inflammation-induced neovessels are the main sites of leukocyte-endothelial cell interactions leading to the development of inflammatory infiltrates [99]. Constitutive [platelet endothelial cell adhesion molecule (PECAM-1), ICAM-1, ICAM-2 and P-selectin] and inducible [E-selectin and vascular cell adhesion molecule 1 (VCAM)] endothelial adhesion molecules for leukocytes are mainly expressed by adventitial microvessels and neovessels within GCA inflammatory infiltrates [100]. Interestingly, the intensity of inducible endothelial adhesion molecule expression (E-selectin and VCAM-1) seems to be correlated with the intensity of the systemic inflammatory response [101]. Neo-vessels also over-express pro-inflammatory cytokines such as IL-32 and IL-33 and may actively participate in shaping the immune inflammatory milieu in GCA arteries [68,72]. An active recognition of endothelial cells seems also to occur in GCA since the demonstration that patients build anti-endothelial-cell antibodies reacting against vinculin, lamin A/C, voltage-dependent anion-selective channel protein 2, and annexin V [101]. A recent study suggested the presence of possible communication pathways between T cells, vascular smooth muscle cells (VSMC) and endothelial cells [10]. In this study, the Authors demonstrated that inflamed temporal arteries from patients with GCA contain a strong gene expression signal for Notch homolog 1, translocation-associated (Drosophila) (NOTCH) receptors and ligands. In physiologic conditions, interactions between Notch receptors and Notch ligands regulate VSMC differentiation and plasticity, mediate VSMC-endothelial cell communication and promote angiogenesis [10]. T cells from patients with GCA have been demonstrated to over-express NOTCH1 receptor and to interact with ligand-expressing DCs and endothelial cells. Interestingly, blockade of Notch-Notch ligand interactions suppressed experimentally induced vasculitis and down-regulating pro-inflammatory pathways in inflamed arteries [10].

1.11. miRNA overexpression in GCA

MicroRNA (miRNAs) are small, non-coding RNAs which regulate the expression of multiple genes by inhibiting mRNA translation and inducing mRNA degradation through base pairing. Recently 6 miRNAs have

been found overexpressed in inflamed TAs from GCA patients compared to non-inflamed, normal TAs: miR146b-5p, -146a, -21, -150, -155, -299-5p [102]. Noteworthy, miR146b-5p, -146a, -21 and -155 have been found overexpressed also in abdominal aortic aneurysms and atherosclerotic plaques indicating an overlap between pathogenetic mechanisms in GCA and other inflammatory cardiovascular diseases. It is actually unknown whether such miRNAs are biomarkers of specific infiltrating immune cell subsets and activated pathways in inflamed TAs and/or have a functional role in GCA pathogenesis. MiR-146a, -21, -155 and -150 can be expressed by specific immune cell subsets [102]. MiR-155 is mainly a pro-inflammatory miRNA, miR-21 can have both pro- and anti-inflammatory activities whereas miR-146a and miR-150 mainly restrain inflammation by negative feedback circuits. Expression of miR-146b-5p, 146a, -21 and -155 is at a downstream point of the activation of Nuclear Factor- κ B (NF- κ B), TLRs and signal transducer and activator of transcription 3 (STAT3) suggesting that these pathways might be involved in GCA pathogenesis. Noteworthy such miRNAs can be induced by cellular senescence and inflammation [102]. Moreover, arterial wall cells such as VSMCs, endothelial cells and adventitial fibroblasts can also express them. MiR-21 is the only miRNA overexpressed in GCA that has documented pathogenic effects on VSMCs, endothelial cells and adventitial fibroblasts, thus emerging as a promising target for the development of novel gene-therapy approaches for GCA. In this regard, local delivery of anti-miR-21 oligonucleotides decreased neointima formation in preclinical models of atherosclerosis [103].

2. Conclusions

The complexity of GCA pathogenesis is greater than first imagined. The senescence of the immune system together with the appearance of ATLOs may drive a series of complex and coordinated interactions between stromal and endothelial cells and innate and adaptive immune cells. The resulting outcome of these complex immune reactions is a complex disease in which different histologic findings and clinical manifestations may occur. The role of VZV, although fascinating, requires further studies to be definitively accepted as a causative agent of the disease.

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