REVIEW

Immunopathogenesis of granulomas in chronic autoinflammatory diseases

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Granulomas are clusters of immune cells. These structures can be formed in reaction to infection and display signs of necrosis, such as in tuberculosis. Alternatively, in several immune disorders, such as sarcoidosis, Crohn's disease and common variable immunodeficiency, non-caseating granulomas are formed without an obvious infectious trigger. Despite advances in our understanding of the human immune system, the pathogenesis underlying these non-caseating granulomas in chronic inflammatory diseases is still poorly understood. Here, we review the current knowledge about the immunopathogenesis of granulomas, and we discuss how the involved immune cells can be targeted with novel therapeutics.

INTRODUCTION

Inflammation is a physiological response of the body to invading pathogens. However, if the inflammatory state is not transient and persists chronically, this can result in irreversible tissue damage.[1] Typical non-infectious causes of chronic inflammation are autoimmune diseases, which are characterized by T-cell and antibody responses to self-antigens. Disorders that are characterized by innate immune responses without obvious autoantibodies are referred to as autoinflammatory diseases.[2] In several autoinflammatory diseases, chronic inflammation can result in the formation of granulomas, which are clusters of immune cells in affected tissues.

The most common cause of all granuloma formation worldwide is tuberculosis.[3] The formation of granulomas in tuberculosis is thought to be a physiological reaction to prevent the systemic spread of the causative pathogen, the mycobacterium.[4] This immune response typically results in a caseating granuloma with signs of necrosis.[5] Many other infectious agents can trigger granuloma formation (Table 1), as well as foreign body material such as beryllium, and inherited defects in neutrophil function (chronic granulomatous disease).[3,6–9] In chronic inflammatory diseases and primary immunodeficiencies with chronic inflammation, the granulomas have not been associated with specific external agents. With the exception of granulomatosis with polyangiitis, these granulomas are non-caseating (Figure 1) and typically observed in patients with sarcoidosis,[10] Crohn's disease[11] and common variable immunodeficiency (CVID).[12]

In recent years, several new insights have been generated into granulomatous inflammation. These new insights might soon be translated to clinical care, as increasing numbers of therapeutic agents targeting various immune pathways are currently tested in clinical trials.[13] Here, we review and discuss recent literature on granulomatous inflammation in sarcoidosis, Crohn's disease and CVID, all chronic inflammatory disorders with similar types of granulomas without a known trigger. We will specifically address the immune components involved in granuloma formation and how these can be used as disease markers and targeted by new therapeutic approaches for chronic autoinflammatory diseases with granuloma formation.

CHRONIC AUTOINFLAMMATORY DISEASES WITH GRANULOMA FORMATION

Sarcoidosis

Sarcoidosis is a multisystem granulomatous disease of unknown etiology. The hallmark of this disease is the presence of non-caseating granulomas affecting multiple organs. It is a rare disease with a worldwide prevalence ranging from 1 to 40 per 100 000 and a peak incidence at 20–39 years of age.[14] The clinical presentation of sarcoidosis is highly variable and dependent on the organs involved. Systemic complaints of fever, weight loss and fatigue are common. About 90% of patients have pulmonary granulomas with frequent involvement of other organs such as lymph nodes, skin, liver, eye, central nervous system and heart.[10] Owing to the high variability in clinical manifestations, it can be challenging to diagnose sarcoidosis. There is no definite test and diagnosis of sarcoidosis is based on three elements: (1) clinical and radiographic manifestations; (2) exclusion of diseases that may present similarly; (3) identification of non-caseating granulomas by histological analysis of tissue.[15] Chest X-ray and computed tomography are the most common used visualization

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Table 1 Overview of infectious and non-infectious diseases with granuloma formation

Category	Disease	Type of granuloma	Localization
Infectious			
Bacterium	Tuberculosis	Caseating necrosis	Lung, extrapulmonary; disseminated
	Brucellosis	Necrotizing and fibrotic	Liver, spleen
	Bartonellosis	Necrotizing	
	Actinomycosis	Non-caseating	Cervicofacial, abdominal, lung
Fungus	Histoplasmosis	Necrotizing	Lung
	Aspergillosis	Necrotizing	Lung
	Candidiasis	Necrotizing with abcesses	Skin
	Cryptococcal disease	Fibrotic with abcesses	Lung
Parasitic	Leishmaniasis	Necrotizing	Skin
	Dirofilariasis	Fibrotic and calcifying	Subcutaneous
	Schistomiasis	Non-caseating	Liver, intestines, bladder
Viral	CMV	Unspecified	Spleen and liver
	EBV	Unspecified	Skin
	Measles	Unspecified	Thyroid gland
Non-infectious with known cause			
Primary immunodeficiency	CGD	Non-caseating	Skin, intestines, liver
Malignancy	Lymphoma	Non-caseating	Lymphatic tissue
	Foreign body	Non-caseating	Tissue with contact to foreign body particle; skin, lung, intestines
Other	Berylliosis	Non-caseating	Lung
Non-infectious with unknown cause			
Chronic inflammatory disease	GPA	Necrotizing	Lung, upper airways
	Sarcoidosis	Non-caseating	Lung, skin, eye, lymph node, liver, CNS, heart
	Crohn's disease	Non-caseating	Intestines, skin, liver, lymph node
Primary Immunodeficiency	CVID	Non-caseating	Lung, lymph node, liver, skin, spleen, intestines

Abbreviations: CGD, chronic granulomatous disease; CMV, cytomegalovirus; CNS, central nervous system; CVID, common variable immunodeficiency; EBV, Epstein–Barr virus; GPA, granulomatosis with polyangiitis. This table provides a non-exhaustive list of causes of granuloma formation. The affected organs are listed from the most commonly involved organ on the left to less common. The information is derived from refs 3,6–9,185–190.

techniques. Radiographic pulmonary manifestations can vary from bihilar lymphadenopathy, pulmonary infiltration or fibrosis.[16] Nuclear techniques, such as the fluorine-18 fluorodeoxyglucose positron emission tomography, can also be used to evaluate extrapulmonary manifestations of sarcoidosis or to find a location for biopsy.[17] Blood tests can provide supportive information for making the diagnosis through detection of high serum levels of angiotensin-converting enzyme or soluble interleukin 2 receptor (sIL-2R), which is a marker for increased activation of T cells.[14,18]

Fortunately, treatment is not necessary in over 50% of patients in whom the disease will resolve in 3 years without medication.[10,14] Patients are only given medication when inflammation leads to organ damage. First-line therapy for sarcoidosis is based on corticosteroids such as prednisone. Second-line treatment comprises immuno-suppressive medication such as methotrexate and azathioprine. For refractory cases, third-line medication is available in the form of biologicals that block tumor necrosis factor-α (TNF-α): infliximab or adalimumab.[19] This approach is successful in ~50% of treated patients in whom the granulomas resolve with no or little remaining organ damage. However, 20–25% of all diagnosed patients develop chronic disease with pulmonary fibrosis.[14] Current therapies target inflammatory pathways and have little effect on fibrosis. This is a major limitation because fibrosis results in increased morbidity and mortality and the need for lung transplantation.[20] The lack of a cure for sarcoidosis underlines the need to find new, effective drugs.[10,14]

Crohn's disease

Crohn's disease is an inflammatory bowel disease.[11] In recent years, the worldwide prevalence of Crohn's disease has been reported to increase, with current estimates in Western countries of 25 to 318 per 100 000.[21] Similar to sarcoidosis, Crohn's disease typically affects young adults, but with a 10-fold higher prevalence. The chronic inflammation in the intestinal tract is thought to result from an interplay of the genetic background, environmental factors, intestinal microbiota and a dysregulated immune system.[22] In Crohn's disease, chronic inflammation can manifest throughout the gastrointestinal tract, mainly affecting the ileum and the colon resulting in abdominal pain and diarrhea with passage of mucus or blood.[11] In addition, subsets of patients show inflammation of the skin, eyes or joints. Diagnosis of Crohn's disease is based on clinical assessment and physical examination of the patient in conjunction with imaging and histopathology of inflamed tissues and with blood tests.[11] Crohn's disease has many overlapping features with ulcerative colitis,[23] the other major variant of inflammatory bowel disease. In contrast to Crohn's disease, inflammation in ulcerative colitis is restricted to the colon and does not result in granuloma formation. When inflammatory bowel disease is suspected, a colonoscopy is performed during which biopsies are taken. The histological finding of a non-caseating granuloma is the most discriminating factor for Crohn's disease.[24] Supporting evidence from laboratory analyses include high C-reactive protein, low hemoglobin and high fecal calprotectin.[11] Furthermore, the majority of patients has detectable serum levels of anti-
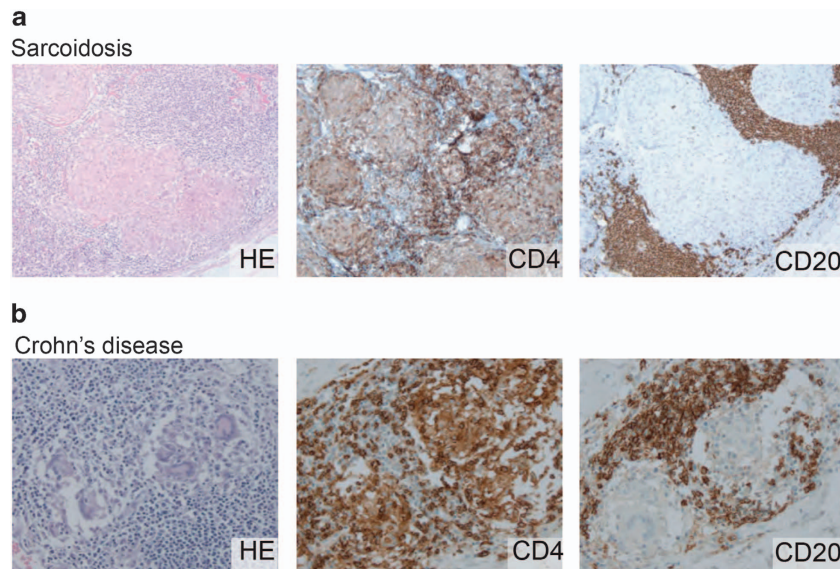
Figure 1 Non-caseating granulomas in Crohn's disease and sarcoidosis. Hemotoxylin and eosin stainings reveal granulomatous structures in a lymph node biopsy of a patient with sarcoidosis (**a**) and a biopsy of the ileum of a patient with Crohn's disease (**b**). Typically, CD4-expressing Th cells are detected in and around the granulomas, whereas CD20-expressing B cells are found to accumulate around the granulomas.[118,130]

Saccharomyces cerevisiae antibodies,[25] or antibodies to the outer membrane porin C of *Escherichia coli* (anti-OmpC).[26] Despite granulomas being a discriminating factor with ulcerative colitis, these structures are only identified in ~ 37% of patients with Crohn's disease.[23] The presence of granulomas is associated with higher rates for surgical bowel resection, indicating that these are an indicator for severe disease.[23] Treatment of Crohn's disease is similar to sarcoidosis and includes corticosteroids, immunosuppressive and biologicals. In spite of the introduction of infliximab, treatment outcomes remain suboptimal with disease control being achieved in only 60% of Crohn's patients,[27] and intestinal complications and the requirement for surgery remain.[11]

CVID with granulomatous complications
CVID is a primary immunodeficiency. It is a rare, heterogeneous disease with a prevalence of 2 to 4 per 100 000 and mean age of diagnosis between 30 and 40 years.[28] Patients suffer from recurrent sinopulmonary infections and to a lesser extent from gastrointestinal infections. The hallmark of CVID is a B-cell defect leading to low or absent levels of immunoglobulins, and can be accompanied by abnormal T-cell responses and cytokine defects. Diagnosis of CVID is made when a patient has severely reduced levels of serum immunoglobulin G (IgG) with low IgM and/or IgA, and fulfills all of the following criteria: (1) onset after 2 years of age; (2) poor or absent vaccination response and (3) exclusion of other causes of hypogammaglobinemia.[29] Despite these commonalities in immunological defects and recurrent infections, CVID represents a heterogeneous group of patients with ranging clinical features that include autoimmunity, granuloma formation and hematological malignancies. These non-infectious complications are associated with high morbidity and early mortality.[30] Previously, only in 2–10% of patients a molecular cause of disease was identified in genes such as *ICOS*, *CD19*, *CD81*, *TNFRSF13C* (encodes BAFFR) and *TNFRSF13B* (encodes TACI).[31–35] However, none of these correlated with the incidence of granulomatous complications in 8–22% of CVID patients.[12] With the recent identification of autosomal-dominant

causes of complex antibody deficiencies and incomplete penetrance of some mutations (e.g. *CTLA4*, *PIK3CD*, *PIK3R1* and *NFKB1*),[36–39] it will become possible to relate granulomas to a genetic cause.

In CVID patients, granulomas most prevalently affect the lungs, followed by lymph nodes, liver, skin and spleen. The presence of granulomas can precede the diagnosis of CVID for years resulting in a potential misdiagnosis of sarcoidosis. However, sarcoidosis patients do not present with recurrent infections or low/absent immunoglobulins, because serum IgG levels are normal or even elevated in sarcoidosis.[40] CVID patients can also present with abdominal complaints, such as chronic diarrhea, weight loss and histological evidence of intestinal inflammation, resulting in an overlap of clinical features with Crohn's disease.[41] CVID patients with granulomas are more frequently affected by other autoimmune manifestations and have a higher morbidity and mortality rate than non-granulomatous patients.[12,42] The primary treatment of CVID is intravenous or subcutaneous immunoglobulin substitution, which is highly effective in reducing the infectious burden.[43] However, this treatment does not ameliorate the non-infectious complications. Conversely, granulomatous inflammation in CVID is treated with similar types of immunosuppressive agents that are used for sarcoidosis and Crohn's disease. The combination of immunodeficiency with inflammation highlights the complicated processes involved in CVID, because it appears contrasting to treat immunocompromised patients with immunosuppressive medication.

While granulomas are the hallmark of disease in sarcoidosis, these are only detected in subgroups of patients with Crohn's disease and CVID. However, the exact incidence of granulomas in these disorders remains unclear and might be underestimated because of sampling errors.[23] Furthermore, granulomas in CVID are often poorly recognized by physicians or upon discovery the patient is mis-diagnosed with sarcoidosis.[12] As granulomatous complications are a predictor for poor disease outcome in CVID[12,42] and a pathognomic feature in Crohn's disease,[44] detection of these inflammatory structures is important in diagnostic workup.
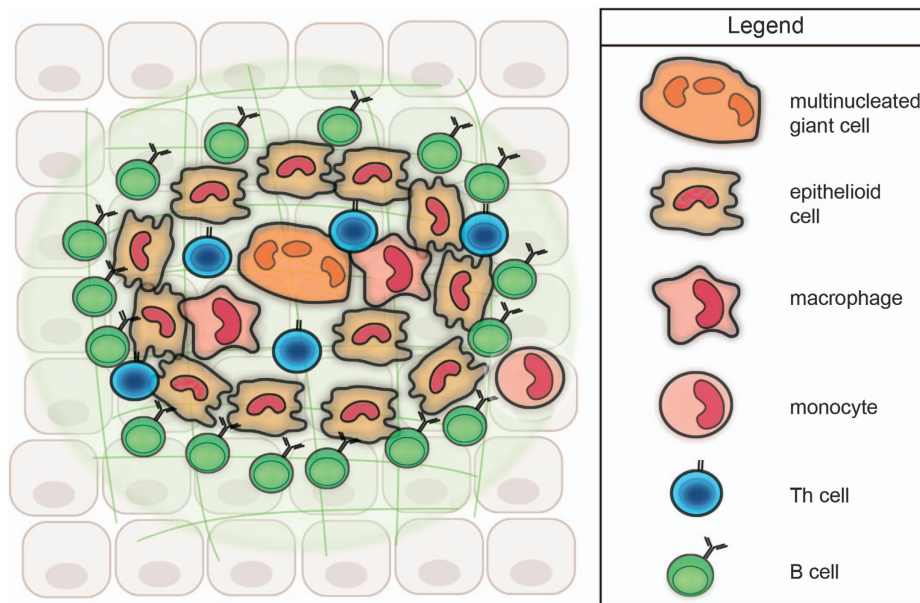
Figure 2 Model of the cellular organization of a non-caseating granuloma. Histology of granulomatous tissue (e.g. in Figure 1) display the presence of macrophages, epithelioid cells and multinucleated giant cells in the core of the granuloma. Th cells are localized in and around the granuloma. B cells are rarely seen in granulomatous structures; however, they are abundantly present around granulomas.[118,130]

KEY PLAYERS IN GRANULOMA PATHOGENESIS

Antigenic triggers

Granulomas are thought to be formed following by a foreign trigger. Therefore, in diseases thus far characterized by non-infectious granulomatous inflammation, the search for a causative agent is still ongoing. In sarcoidosis there is a particular interest in finding the responsible trigger. An increased number of sarcoidosis cases was reported in rescuers after the terrorist attack on the World Trade Center in New York,[45] suggesting an external antigenic cause. Mycobacteria and *Propionibacterium acnes* are of specific interest, because DNA of these antigens was found in granuloma material from sarcoidosis patients with numbers ranging from 0 to 9% for *Mycobacterium tuberculosis* and 79 to 100% for *Propionibacterium acnes*.[46] However, the causality of one single pathogen is debatable with such diverse pathogens being proposed.[10] Antigenic agents have also been suggested to trigger granuloma formation in Crohn's disease, mainly because of the associated defective bacterial clearance by autophagy. Polymorphisms in genes involved in autophagy have been reported,[47] the mechanism by which cells degrade and recycle of cellular components. In Crohn's disease this leads to the impaired capacity to handle pathogens by specialized intestinal epithelial cells, Paneth cells.[48] Furthermore, the presence of anti-*Saccharomyces cerevisiae* antibodies and anti-OmpC antibodies are suggestive of fungal or bacterial triggers of granuloma formation.[25,26] Finally, the high prevalence of *Mycobacterium avium* in blood and tissue suggested that, similar to sarcoidosis, granulomas in Crohn's disease were formed in response to mycobacteria.[49,50] This theory is considered controversial, because *M. avium* is not typically pathogenic in humans and treatment of patients with anti-mycobacterial agents was proven ineffective.[51] An antigenic driver for persistence of granulomas in CVID is unlikely, because these patients are regularly treated with antibiotic or anti-fungal drugs, and these do not effectively resolve this type of inflammation.[12,52] Yet, a high prevalence of human herpesvirus type 8 is reported in granulomatous or lymphocytic interstitial lung disease patients (67%) as compared with the low prevalence of 4.8% in patients with CVID without granulomatous or lymphocytic

interstitial lung disease. Human herpesvirus type 8 infection might therefore contribute to the poor prognosis of patients with granulomatous CVID.[53]

In conclusion, there is no unambiguous evidence for specific causal factors that trigger non-infectious granulomatous inflammation. It is evident that the immune system drives tissue-destructive inflammation, but it remains to be determined if certain infectious or non-infectious particles are prone to trigger formation or persistence of granulomas.

Macrophages

Macrophages are immune cells that are specialized in clearing of degraded extracellular substances through phagocytosis. These specialized immune cells are derived from circulating monocytes and are typically found in granulomas (Figure 2). Macrophages are thought to be one of the first cell types to migrate into affected tissue to clear debris and recruit other immune cells.[54] An important cytokine produced by macrophages is TNF-α, which induces vasodilation and thereby facilitates the infiltration of monocytes and lymphocytes. Macrophages also release other proinflammatory cytokines such as IL-1, IL-6, IL-12 and IL-23. Together with TNF-α, these cytokines promote leukocyte infiltration and T-cell activation, while inhibiting regulatory T cells (Tregs) and T-cell apoptosis.[54] These activated macrophages are important in cell-mediated inflammation seen in granulomas, yet they also induce tissue damage. Polarization of macrophages mirrors the T-helper immune response status. Macrophages can acquire different functionalities in response to local triggers.[55] One definition to describe the activated state of macrophages is the classical M1 and alternative M2 activation. M1 macrophages are activated by Toll-like receptors and interferon-γ (IFN-γ) produced by Th1 cells.[56,57] M2 macrophages are activated through IL-4 and IL-13 and secrete extracellular matrix components promoting tissue remodeling.[56,57] Inflamed tissue in patients with Crohn's disease predominantly contain M1 macrophages,[58] and these contribute to the intestinal inflammation by disrupting the epithelial barrier in Crohn's disease.[59] A similar M1 polarization was seen in
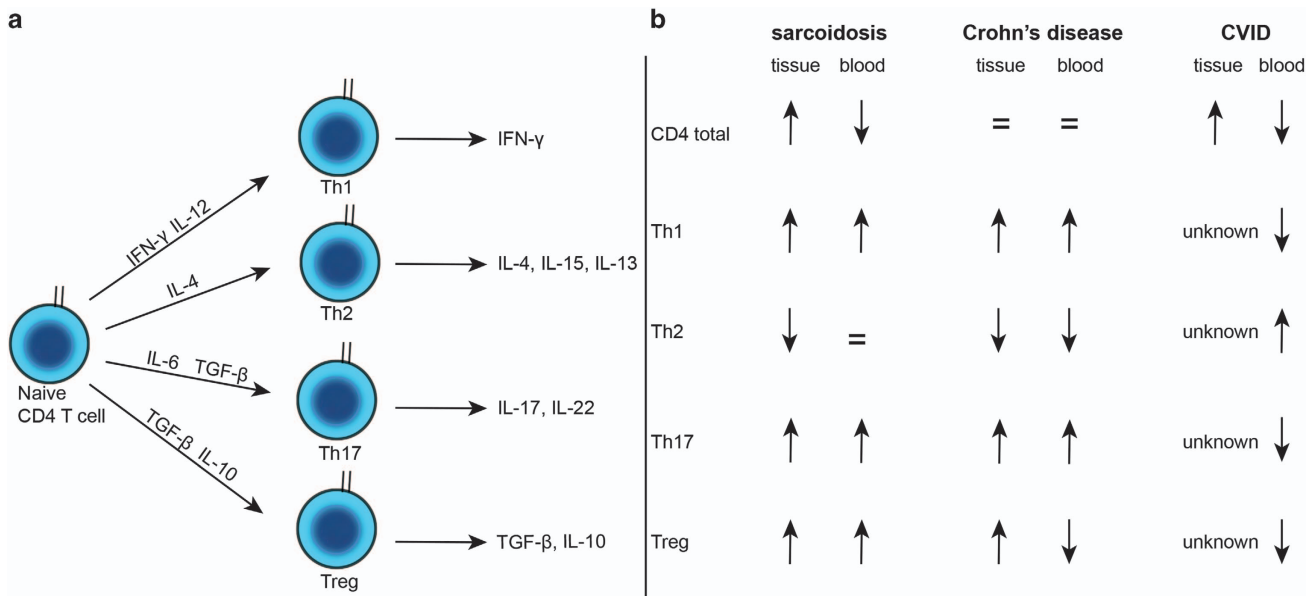
Figure 3 Involvement of CD4+ Th cell subsets in three chronic granulomatous inflammatory diseases. (a) Model of Th cell maturation into Th1, Th2, Th17 and Treg subsets. Key cytokines are depicted. (b) Summary of observations on total CD4+ Th as well as Th1, Th2, Th17 and Treg subsets in tissue and blood of patients with sarcoidosis, Crohn's disease and CVID.[87,88,111,112,177–184]

alveolar macrophages of patients with sarcoidosis.[60] Interestingly, an M2 polarization has been reported in other interstitial lung diseases with fibrosis. This is in line with an M2 polarization in a Th2 environment that has been observed in neurosarcoidosis with myofibrisosis,[61] and in fibrotic intestinal lesions of patients with Crohn's disease.[62] These studies suggest an M1 activation predominantly in the acute proinflammatory granulomatous inflammation with a possible shift towards M2 macrophages in fibrotic processes.

Stimulated macrophages can further mature into epithelioid cells that are elongated and resemble epithelial cells. Epithelioid cells appear to lose their phagocytic function and shift to more secretory capacities.[63,64] However, to our knowledge, it remains unclear what soluble factors these epithelioid cells produce. Epithelioid cells can fuse together and create compact aggregations, which are called multinucleated giant cells.[65] In contrast to epithelioid cells, these multinucleated cells are capable of phagocytosis and cytokine secretion, especially IL-1, TNF-α and tumor growth factor-β.[66]

Our understanding of TNF-α and its role in granuloma integrity is mostly based on tuberculosis animal models.[67,68] In the absence of TNF, primary granulomas can still be formed. However, granulomas appeared disorganized.[67,68] Furthermore, a loss of TNF signaling disrupts already formed granulomas. This could, in part, be due to impaired lymphocyte recruitment and activation, in which TNF-α also has a major role.[67]

Several abnormalities in monocyte and macrophage function have been reported in sarcoidosis, CVID and Crohn's disease, and these might contribute to the chronic inflammation and granuloma formation. Specifically, monocytes in patients with sarcoidosis and Crohn's disease have an increased ability to form multinucleated cells.[69,70] Furthermore, cultured alveolar macrophages of patients with sarcoidosis spontaneously produce more proinflammatory cytokines, including TNF-α, compared with controls,[71] and these higher levels were associated with progressive disease.[72] TNF-α production was also found to be increased in monocytes of patients with CVID.[73] The TNF 488A allele leads to higher TNF production and is strongly positively associated with granulomatous CVID.[74] Furthermore, 82% of TNF 488A allele-negative patients were IL-10 a-t-a allele positive, leading to lower IL-10 production resulting in a more proinflammatory TNF environment. Taken together, these two genetic variants seem to promote a cytokine shift contributing to an inflammatory environment leading to granulomatous complications.[75] The intestinal microbiota can also affect the inflammatory environment. Intestinal macrophages of patients with Crohn's disease produced more proinflammatory cytokines such as TNF-α after stimulation with commensal bacteria,[76] whereas reduced levels of proinflammatory cytokines were reported in response to E. coli.[77–79] Furthermore, E. coli is able to survive and replicate in intestinal macrophages in patients with Crohn's disease, is present in granulomas and can induce granuloma formation in vitro.[80–82] Owing to this apparent decreased macrophage function, it has been proposed that Crohn's disease should also be considered a primary immunodeficiency.[83,84]

T cells

The inflammatory mediators produced by macrophages in affected tissue trigger the recruitment of additional immune cells, especially CD4+ Th cells (Figures 1 and 2). Th cells are important mediators of immune responses and are thought to organize the granulomatous structure together with the already present macrophages. Traditionally, Th cells were divided in Th1 and Th2 subsets, and the Th cells in granulomatous tissue were assumed to be type 1 cells that produce IL-2 to induce T-cell proliferation and the accumulation of effector T cells. However, with the more recent detection of other subsets such as Th17 cells and Tregs, the concepts of Th-mediated inflammation have changed.[85]

Naive Th cells have the ability to differentiate in a particular subset through a specific cytokine milieu. The major subsets are Th1, Th2, Th17 and Tregs that are defined by their cytokine profiles and distinct effector functions (Figure 3). Th1 cells develop in the presence of IFN-γ and IL-12 and protect against intracellular pathogens through the production of IFN-γ and the resulting macrophage activation.[54,85] Upregulation of cytokines promoting Th1 differentiation have been reported in sarcoidosis: IL-2, IL-12, IL-15 and IL-18.[86] Th cell involvement in sarcoidosis is underpinned by the typical CD4

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T-cell lymphopenia in peripheral blood in combination with CD4 T-cell infiltrates at the site of inflammation, such as in bronchoalveolar lavage fluid.[87,88] Despite these signs of local T-cell hyperactivity, the typical diminished cutaneous response to tuberculin is suggestive of T-cell anergy in non-granulomatous tissue.[89] CD4 T-cell anergy in these patients is likely due to chronic stimulation and results from reduced availability of G proteins,[90] and reduced nuclear factor-κB capacity of these cells.[91]

Patients with Crohn's disease show overexpression of IL-12 in intestinal tissue leading to increased production of IFN-γ.[92,93] Still, total blood CD4 T-cell numbers as normal, and even an expansion of CD4 memory T cells has been observed in patients with active Crohn's disease.[54] The hyperactive state of inflammation in Crohn's disease is further illustrated by mucosal T-cell proliferation and expansion with resistance to apoptosis.[94] Unlike sarcoidosis and Crohn's disease, patients with granulomatous CVID have low levels of total T cells and naive CD4 T cells.[74] This decrease could be related to the immunodeficiency and result from increased T-cell turnover and apoptosis or decreased thymic output. It remains unclear whether this decrease also distinguishes granulomatous inflammation from Crohn's disease and sarcoidosis or it is the result of migration of T cells from circulation to the affected tissue.

In addition to Th1 responses, other Th subsets have been implicated in chronic inflammation. It is thought that the initial Th1 response during acute granulomatous inflammation shifts to a Th2 response in response when this becomes chronic. The production of Th2 cytokines can activate and stimulate fibroblasts and thereby contribute to fibrosis.[20]

More recently, Th17 have been shown to be disruptive in chronic inflammatory diseases.[95] Th17 cells are generated in the presence of IL-6 and tumor growth factor-β, and in turn produce IL-17 and IL-22 that are major factors in responses against extracellular pathogens and fungi (Figure 3). IL-17 was proposed to be a key mediator of inflammation in rheumatoid arthritis, yet anti-IL-17 therapy with secukinumab was not effective.[96] Therefore, the exact role of Th17 cells in inflammatory disorders is not clear and information is mostly based on animal models. IL-17 overexpression leads to tissue damage in different organs such as lungs, intestines, joints and brain.[97] Th17 cells have the ability to change to a Th1 phenotype enabling cells to produce both IFN-γ and IL-17 referred to as Th1/Th17 cells.[98] The plasticity of Th17 cells enables to further enhance inflammation either directly through the coproduction of IL-17 and IFN-γ or through providing help in the generation of new pathogenic Th1 cells.[99] Moreover, Th17 cells in mouse models have recently been shown to adapt into a regulatory phenotype with a change in transcriptional profile and regulatory capacities.[100]

In both sarcoidosis and Crohn's disease IL-17 expression is increased in inflammatory tissue, concomitant with an increase of Th17 cells in the peripheral blood.[22,101] In contrast, CVID patients have low Th17 cell numbers in their peripheral blood, which is associated with higher numbers of CD21^{low} B cells and lower numbers of memory B cells.[102] The presence of an expanded CD21^{low} B-cell population in CVID patients is associated with higher incidence of non-infectious complications.[103] The concomitant decrease in Th17 cells is suggestive of a combined defect in B and T cells in this subset of CVID patients. The nature of this defect remains to be determined and could be B- or T-cell intrinsic or arise from impaired regulation of Th maturation.[102]

Tregs are important to dampen immune responses and thereby maintain a physiological immune homeostasis and self-tolerance.[104] Naive T cells can mature into Tregs through the expression of the transcription factor Forkhead box p3 (FoxP3) in the context of tumor growth factor-β, subsequently exerting immune regulatory functions through production of tumor growth factor-β and IL-10.[85] Tregs became an intensively studied cell population after it was reported that CD4$^+$CD25$^+$ depletion in mice resulted in a variety of autoimmunity including gastrointestinal involvement.[105] Furthermore, patients with immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome, a genetic disorder caused by mutation in the *FOXP3* gene, are affected by excessive gastrointestinal autoimmunity.[106]

In patients with sarcoidosis, higher frequencies of Tregs have been reported in both peripheral blood and bronchoalveolar lavage fluid with accumulation of Tregs in the vicinity of granulomas.[107] These Tregs inhibited T-cell proliferation, yet several groups confirmed a decreased suppressor function on CD4$^+$ cells.[107–109] Moreover, Tregs from patients with active sarcoidosis were not able to suppress granuloma formation in an *in vitro* model, whereas Treg cells from healthy controls were.[110] However, it remains unclear whether Tregs were defective or were merely exhausted as a result of the continuous inflammation. Patients with active Crohn's disease have decreased Treg numbers in the blood, whereas these are increased in the intestinal mucosa.[111,112] The anti-inflammatory function of Tregs is likely to be intact as they have preserved suppressor function,[112] and are able to inhibit effector T-cell responses.[111] However, it has been postulated that effector T cells in the lamina propria are unresponsive to the inhibiting effects of Tregs, implicating a contributing factor to the chronic inflammatory response.[111] Treg function in CVID has been less well documented, yet decreased levels of Tregs in blood of CVID patients were specifically seen in patients with autoimmune complications.[113] Importantly, Tregs require the inhibitory receptor cytotoxic T-lymphocyte-antigen 4 (CTLA-4) for suppressive function, and mutations in *CTLA4* underlie an immunodeficiency in which Tregs have reduced suppressive function. These patients often present with granulomatous inflammation and intestinal inflammation with similarities to Crohn's disease.[39,114,115] As decreased CTLA-4 expression on Tregs has also been reported in patients with sarcoidosis, it is possible that defects in CTLA-4 and Treg function contribute to granuloma formation in autoinflammatory diseases.[116]

B cells

The main focus in granulomatous inflammation has previously been directed to macrophage and T-cell dysfunction. However, in addition to macrophages and T cells, B-cell infiltrates are present in granulomatous tissue of patients with tuberculosis.[117] Furthermore, several studies showed that numerous B cells surround granulomas in affected tissues from patients with sarcoidosis as well as Crohn's disease (Figure 1).[118–120] These B cells are likely to be essential for the development of granulomas as indicated by two findings: first, patients with CVID can develop granulomas, whereas patients with X-linked agammaglobulinemia do not.[121] CVID and X-linked agammaglobulinemia patients both have an antibody deficiency due to B-cell dysfunction, but mature B cells are completely absent in X-linked agammaglobulinemia.[122] Second, in a mouse model of oil granulomas the absence of T cells did not affect the ability of granuloma formation, whereas granulomas were not formed in the absence of B cells.[123] Traditionally, B cells are regarded as the antibody-producing cells of the immune system. While this is a major function of B cells, it has become clear that B-cell development is a complicated process with many B-cell subsets and functions involved. Other B-cell functions include the ability to act as antigen-presenting cells, costimulate T cells, have regulatory effects and produce cytokines that direct Th subset maturation.[124] These insights, together with novel

possibilities to target B cells with biologicals, provide a strong rationale to investigate the role of B cells in granulomatous inflammation.

Some recent insights have been generated into B-cell abnormalities in sarcoidosis and Crohn's disease. Patients with sarcoidosis carry reduced numbers of IgM, IgG and IgA memory B cells and plasma cells in blood with the exception of CD27⁻IgA⁺ memory B cells.[118,125,126] Despite their reduced numbers, levels of somatic hypermutations in Ig gene transcripts of these cells were increased, suggestive of chronic activation.[118] This is potentially related to serum B-cell activating factor (BAFF), a critical factor for mature B-cell survival of which the levels are increased in sarcoidosis patients with active disease.[125,126] Furthermore, the levels of nuclear factor-κB transcription factors in B cells are reduced and potentially affect B-cell responses and proliferation.[127] How these B-cell abnormalities affect the formation of persistence of granulomas still needs to be determined, yet combined these results do suggest a disturbed B-cell homeostasis.

Patients with Crohn's disease also display reductions in blood IgM memory B-cell numbers. In contrast to sarcoidosis, they have normal numbers of Ig class-switched memory cells plasma cells.[128,129] In addition, transitional B cells and anergic CD21low B cells were found to be expanded.[130] Expansions of CD21low B cells are an indication of chronic activation,[131] as were the observed increased levels of somatic hypermutions in Ig gene transcripts.[130] It remains unclear if the decline in memory B cells is the result of impaired generation from, for example, the splenic marginal zone, or from their specific recruitment to granulomatous tissue.

CVID is characterized by hypogammaglobinemia and all patients have reduced blood plasma cells, which in many patients is accompanied by memory B-cell defects.[103] Furthermore, in subgroups of patients, expansions of transitional B cells, as well as CD21low B cells, have been identified. Many of these abnormalities have formed the basis of flow cytometry-based classifications.[103] However, B-cell phenotypes do not seem to correlate well with severity of disease or non-infectious complications. Yet, granulomatous complications are found to be associated with lower numbers of Ig-switched memory B cells.[52,103] Still, it remains unclear if these reductions are related to the immunodeficiency or the result of migration towards the sites of granulomatous inflammation. Patients with mutations in *ICOS* (inducible T-cell costimulator) and *TACI* gene can develop granulomatous complications and autoimmunity in general.[132] These genes are involved in different pathways of B-cell survival and T-cell-dependent or -independent antibody responses. With the implementation of whole-exome sequencing, the genetics of CVID unravels rapidly. Possibly, this will provide better insights into affected processes and will help to dissect the mechanisms that, when impaired, result in granuloma formation.

In addition to a role in ongoing inflammation, B cells might also function to dampen or restrict inflammatory processes. Subsets of B cells are capable of production of IL-10, and these regulatory B cells could therefore dampen the ongoing immune response. This function is illustrated by mouse models of colitis, in which B cells were found to ameliorate intestinal inflammation.[133]

Although it remains unclear how B cells contribute to disease pathogenesis, the common signs of chronic activation of B cells in granulomatous autoinflammatory diseases is suggestive of their role in ongoing inflammation. The systemic B-cell abnormalities could provide good markers for disease and treatment monitoring. Moreover, disease-specific abnormalities could provide more insight into pathogenesis and starting points for novel therapeutic approaches.

THERAPEUTIC IMPLICATIONS

Remission or fibrosis?

In many patients granulomas persist and lead to organ damage due to fibrosis. Fibrosis is therefore a common problem in sarcoidosis and Crohn's disease.[14,129] The impact of granulomas on permanent organ damage in CVID patients is currently unknown due to the complications of recurrent respiratory infections that lead to bronchiectasis in 23% of patients.[28] Despite fibrosis leading to increased morbidity and mortality,[20] to date, therapies targeting inflammatory pathways do not resolve or delay the process. Moreover, it is not yet possible to identify which patients will develop fibrotic complications.[10] Therefore, exploring fibrotic pathways may lead to new and much needed therapies to prevent irreversible organ damage.

Mechanism of current therapies

First- and second-line medication to treat patients with chronic inflammatory disease are corticosteroids and immunosuppressives such as methotrexate and azathioprine. Corticosteroids have anti-inflammatory properties through the inhibition of leukocyte migration and proinflammatory cytokine production (esp. TNF-α and IFN-γ).[134] Methotrexate inhibits the purine metabolism and azathioprine purine synthesis, which both lead to decreased lymphocyte proliferation and cytokine release.[135] While these therapies are administered to suppress proinflammatory cytokines by inhibiting T-cell responses, these immunosuppressive drugs also affect the B-cell compartment.[136,137]

With the introduction of biological therapies, a third line of treatment has become available, of which TNFα blockers are most notable. The most widely used TNFα blockers are antibodies against TNFα (infliximab and adalimumab), which have proven to be effective in Crohn's disease and sarcoidosis (Figure 4).[138,139] This treatment specifically disrupts the granuloma structure. As this can result in reactivation of latent tuberculosis, all patients need to be intensively screened for tuberculosis before treatment with TNFα blockers.[140] TNF blockers infliximab and etanercept have proven to be beneficial in some patients with granulomatous CVID.[52,141] Etanercept is a recombinant TNFα receptor fused to an Ig constant region and is often used to treat RA.[142] Importantly, etanercept is not effective in sarcoidosis and Crohn's disease, and can even lead to increased disease activity in these disorders.[143,144] This might be related to its different biological properties as opposed to anti-TNF antibodies: (1) etanercept binds only to soluble trimeric and not monomeric soluble TNF-α; (2) etanercept has low affinity to transmembrane TNF;[145] (3) etanercept binds to both TNF-α and lymphotoxin-α, a cytokine that is crucial for secondary lymphoid organ development, IgA regulation and T-cell gut homing.[146] These abilities could explain the reduced effectivity of etanercept in Crohn's disease and sarcoidosis, as well as observed disease complications. Treatment with TNFα blockers also affects the blood B-cell compartment in patients with Crohn's disease and sarcoidosis.[129,130,147] It remains to be determined if this is an indirect effect following modulation of inflammation or if this is through direct binding to TNFRII that is expressed on B cells.

Targeting of T cells in granulomatous diseases has yielded mixed results. A clinical trial for the treatment of patients with Crohn's disease with abatacept was ineffective.[148] Abatacept is a recombinant fusion protein of CTLA4 with an immunoglobulin. CTLA-4 inhibits T-cell activation by binding to CD28 on T cells. Abatacept has shown beneficial effects in RA patients,[149] and in animal models of intestinal inflammation. These results illustrate that, in spite of unraveling underlying immune mechanisms, translation into effective therapies for human autoinflammatory disease remains challenging.
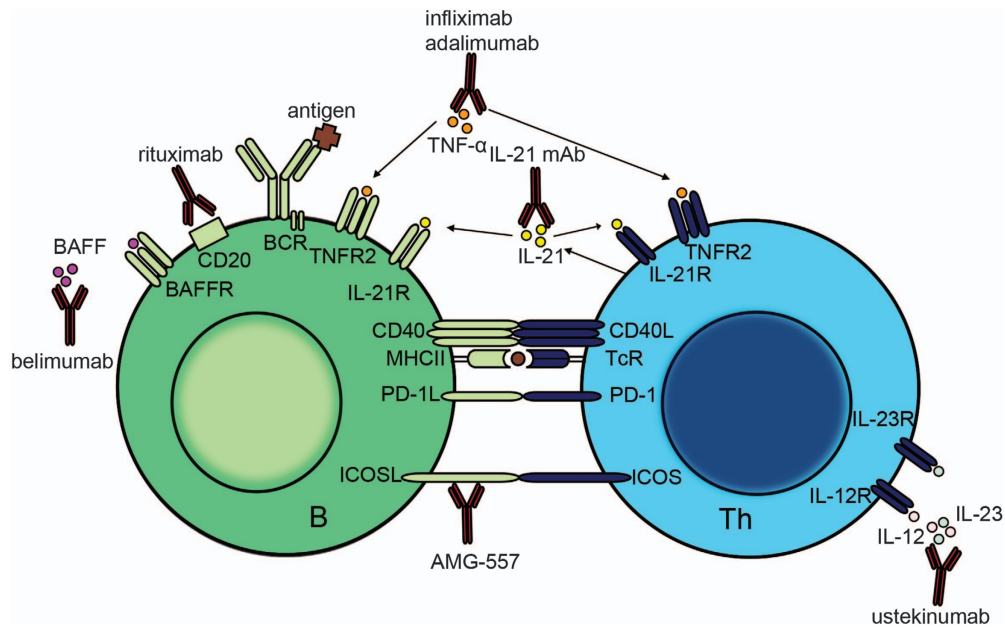
Figure 4 Novel therapeutics that are currently used or in trial for treatment of granulomatous autoinflammatory diseases that target B and/or T cells. Indicated are monoclonal antibodies that specifically target T cells (ustekinumab), or B cells (rituximab and belimumab). Anti-TNFα (infliximab, adalimumab) and anti-IL-21 monoclonal antibodies block cytokines that affect both B and T cells. Finally, AMG-557 targets ICOSL and affects the B–T-cell interaction: BCR, B-cell receptor; mAb, monoclonal antibody; MHCII, major histocompatibility complex class 2; TcR, T-cell receptor.

Targeting of Th17 responses have also been studied. However, blocking IL-17 with secukinumab was ineffective in patients with Crohn's disease,[150] whereas treatment with brodalumab, an anti-IL-17 receptor monoclonal antibody, even resulted in exacerbation of Crohn's disease.[151]

Ustekinumab, a monoclonal antibody against both IL-12 and IL-23, resulted in a clinical response in patients with refractory Crohn's disease[152] and is currently implemented in patients who are resistant to TNFα blockers.[153] However, ustekinumab did not show therapeutic efficacy in sarcoidosis patients.[154]

Patients with Crohn's disease do show a good response to treatment with vedolizumab, a humanized monoclonal antibody that binds to integrin $\alpha_4\beta_7$.[155] As $\alpha_4\beta_7$ specifically mediates gut homing, it can selectively inhibit intestinal inflammation. Because granulomas in patients with sarcoidosis and CVID more frequently present in other tissues than the gut, vedolizumab is likely to have limited effects in these diseases.

Targeting of B cells with rituximab has shown promising results in granulomatous CVID.[52] Rituximab is a humanized anti-CD20 antibody that depletes all naive and memory B cells.[156] The efficacy of rituximab in sarcoidosis is still unclear: several case reports show proven effectivity; however, in one small prospective study with 10 patients, only 5 of them showed a marginal ($>5\%$) improvement of respiratory function.[157] In contrast, a patient with Crohn's disease displayed disease exacerbation following treatment with rituximab, implying a protective role for B cells in Crohn's disease.[158] These different outcomes of rituximab treatment highlight the complexity of the underlying inflammatory processes.

New targets for treatment with biologicals
New therapies are in high demand for refractory patients with chronic inflammatory disorders. As new therapeutic targets become evident and new biologicals may become available in the coming years, we propose therapeutic candidates involving the B and/or T cells (Figure 4).

IL-21 is a cytokine produced by Th cells and stimulates B and T cells through the IL-21 receptor. Increased levels of IL-21 have been reported in inflamed tissue of Crohn's disease patients, with infliximab inducing a downregulation of IL-21.[159] IL-21 is also implicated in the immunopathogenesis of RA and therefore treatment with a monoclonal antibody binding to IL-21, NNC0114-0005, is currently being tested in these patients.[160] When safety and efficacy is proven in RA, this treatment could be translated into other inflammatory disorders such as Crohn's disease. However, caution should be taken because genetic defects in *IL-21* gene were recently reported to cause a severe CVID-like disorder[161] that manifests with early-onset inflammatory bowel disease.[162]

Interestingly, the key in therapy might lie in improving T-cell function by targeting of the inhibitory receptor programmed death-1 (PD-1).[163] In patients with sarcoidosis, PDL-1 expression is increased on T cells in granulomatous tissue, and the number of PD-1-expressing Th cells in blood are increased.[164] As a downregulation of PD-1 on CD4 cells was seen in patients with spontaneous clinical resolution, blocking the PD-1/PD-1L pathway could be a therapeutic target.[164] A variety of malignancies also show upregulation of the PD-1/PD-1L pathway and currently several antibodies against PD-1, such as pembrolizumab and nivolumab, are being tested in the treatment of solid and hematological malignancies.[165] Still, a cautious approach is warranted as sarcoidosis-induced disease also has been reported by the use of pembrolizumab in a patient with sarcoma.[166] This case could be explained by the enhanced CD4 T-cell proliferation that was reported when PD-1 was blocked, which could lead to a Th1 proinflammatory response that is also observed in sarcoidosis.

ICOS and ICOSL are important factors in adaptive immunity through B–T-cell interaction and genetic defects in ICOS have been reported to result in adult onset CVID.[31] Moreover, *ICOSL* gene polymorphisms are associated with Crohn's disease,[167] and increased

ICOS expression was reported on Tregs in patients with sarcoidosis.[168] A monoclonal antibody targeting ICOSL, AMG-557, has been developed and is currently undergoing the first trial in systemic lupus erythematosus.[169] While treatment targeting the ICOS/ICOSL pathway is still under development, it is a potential target of interest for granulomatous inflammatory diseases, because ICOS/ICOSL is an implicated pathway in both sarcoidosis and Crohn's disease. Moreover, targeting this costimulatory pathway affects both T- and B-cell activation without their cellular depletion.[169]

Finally, alternative approaches to target B cells are promising therapeutics. Currently, belimumab, a monoclonal antibody targeting BAFF, is currently implemented in the treatment of systemic lupus erythematosus. BAFF is a cytokine produced mainly by macrophages, and it is essential for mature B-cell survival.[170] Especially autoreactive B cells are dependent on high BAFF levels.[171] As patients with active sarcoidosis and CVID display increased BAFF levels, it likely contributes to disease pathogenesis.[102,125,126] Thus, belimumab might be more effective than rituximab through stronger effects on pathogenic B cells.[170]

Treatment monitoring

With the increasing possibilities for biological therapies, it becomes important to determine which patient should benefit most from which drug. While several agents can be efficacious in patients, there are still subgroups of patients that have refractory disease.[138,139] For example, only 60% of patients with Crohn's disease achieved short-term disease control.[27] Starting a patient on ineffective therapy can be expensive and will delay the start of a potentially effective treatment with the possibility of disease exacerbation. Thus, there is a need for biomarkers that can predict therapy outcome before the start of treatment or shortly after.

Therapeutic drug monitoring for infliximab has been extensively studied. Measurements of serum trough levels of infliximab have become standard in diagnostics because low drug levels resulting from the formation of antibodies against infliximab can hamper therapy success.[172] Treatment monitoring through immunological tests are another option. Specifically, quantification of serum soluble IL-2R levels is routinely used for patients with sarcoidosis,[18] because it correlates with pulmonary function tests and with local disease activity as visualized by fluorine-18 fluorodeoxyglucose positron emission tomography scan.[173,174]

Quantification of serum BAFF levels could be a good biomarker as it is elevated in chronic active sarcoidosis.[125,126] Specific lymphocyte subsets could also act as potential biomarkers for therapy. A restoration of the relative numbers of peripheral blood Tregs has been reported in both sarcoidosis and Crohn's disease in patients responding well to infliximab.[175,176] Furthermore, successful infliximab therapy in Crohn's disease resulted in a normalization of IgM memory B-cell numbers.[129,147] Therefore, with increasing knowledge about the specific immune dysregulation seen in these inflammatory diseases, immune monitoring by analysis of specific lymphocyte subsets are potential biomarkers and improve treatment response rates for patients.

CONCLUSIONS

Granulomatous inflammation is a complex interplay between mature macrophages, Th cells and B cells. Generally, our understanding of the immune system is improving and fortunately biologicals that block TNF-α have become available. Yet, the complexity in chronic inflammatory diseases is illustrated by numerous failed drug trials, while refractory disease make new therapeutics for chronic inflammation much needed. A translational approach towards basic immunology and advances in other immune-mediated diseases remain necessary to improve treatment options for refractory patients. Specifically, cross-disciplinary studies into granulomatous inflammation in various disorders could yield new insights. Studies into granuloma formation in genetically defined immunodeficiencies can provide candidate pathways, whereas insights into immune dysregulation in sarcoidosis and Crohn's disease can provide immunological markers to identify CVID patients at risk for granulomatous complications. Recent insights into disease pathogenesis and the potential involvement of B cells open new avenues for treatment and treatment monitoring. In particular, patients with granulomatous inflammatory disease could benefit from targeting B cells or B–T-cell interactions with new therapeutics.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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