**Review**

**Immunopathogenesis of granulomas in chronic autoinflammatory diseases**

Wilhelmina Maria Cornelia Timmermans\textsuperscript{1,2,3}, Jan Alexander Michael van Laar\textsuperscript{1,2}, Petrus Martinus van Hagen\textsuperscript{1,2} and Menno Cornelis van Zelm\textsuperscript{3}

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.

Clinical & Translational Immunology (2016) 5, e118; doi:10.1038/cti.2016.75; published online 16 December 2016

**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.\textsuperscript{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.\textsuperscript{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.\textsuperscript{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.\textsuperscript{4} This immune response typically results in a caseating granuloma with signs of necrosis.\textsuperscript{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).\textsuperscript{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,\textsuperscript{10} Crohn’s disease\textsuperscript{11} and common variable immunodeficiency (CVID).\textsuperscript{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.\textsuperscript{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.

**CHROMIC 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.\textsuperscript{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.\textsuperscript{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.\textsuperscript{15} Chest X-ray and computed tomography are the most common used visualization

---

\textsuperscript{1}Department of Internal Medicine, Erasmus MC, University Medical Center, Rotterdam, The Netherlands; \textsuperscript{2}Department of Immunology, Erasmus MC, University Medical Center, Rotterdam, The Netherlands and \textsuperscript{3}Department of Immunology and Pathology, Central Clinical School, Monash University, Melbourne, VIC, Australia.

Correspondence: Dr MC van Zelm, Department of Immunology and Pathology, Central Clinical School, Monash University, Level 6, Burnet Centre, 89 Commercial Road, Melbourne, VIC 3004, Australia.

E-mail: menno.vanzelm@monash.edu

Received 25 June 2016; revised 11 November 2016; accepted 12 November 2016
Clinical & Translational Immunology

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                                |
|                              | Cryptococcal disease | Fibrotic with abscesses | Lung                                |
| Parasitic                    | Leishmaniasis      | Necrotizing             | Skin                                |
|                              | Dirofilariasis     | Fibrotic and calcifying | Subcutaneous                        |
|                              | Schistosomiasis    | Non-caseating           | Liver, intestines, bladder          |
| Viral                        | CMV                | Unspecified             | Spleen and liver                    |
|                              | EBV                | Unspecified             | Skin                                |
|                              | Measles            | Unspecified             | Thyroid gland                       |
| Non-infectious with unknown cause | CGD                | Non-caseating           | Skin, intestines, liver             |
|                              | Lymphoma           | Non-caseating           | Lymphatic tissue                    |
|                              | Foreign body       | Non-caseating           | Tissue with contact to foreign body particle; skin, lung, intestines |
|                              | Berylliosis        | Non-caseating           | Lung                                |
| Non-infectious with unknown cause | 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 biliar lymphadenopathy, pulmonary infiltration or fibrosis. 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. 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. Fortunately, treatment is not necessary in over 50% of patients in whom the disease will resolve in 3 years without medication. 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 immunosuppressive 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. This approach is successful in ~50% of 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. 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. The lack of a cure for sarcoidosis underlines the need to find new, effective drugs.

Crohn’s disease

Crohn’s disease is an inflammatory bowel disease. 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. 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. 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. 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. Crohn’s disease has many overlapping features with ulcerative colitis, 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. Supporting evidence from laboratory analyses include high C-reactive protein, low hemoglobin and high fecal calprotectin. Furthermore, the majority of patients have detectable serum levels of anti-
Crohn's disease is suboptimal with disease control being achieved in only 60% of patients.28 Patients suffer from recurrent infections, mainly 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 fulfill 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 hypogammaglobulinemia.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.33 Furthermore, granulomas in CVID are often poorly recognized by physicians or upon discovery the patient is misdiagnosed with sarcoidosis.12 As granulomatous complications are a predictor for poor disease outcome in CVID12,42 and a pathognomonic feature in Crohn’s disease,14 detection of these inflammatory structures is important in diagnostic workup.
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, 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. However, the causality of one single pathogen is debatable with such diverse pathogens being proposed. 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, 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. Furthermore, the presence of anti-Saccharomyces cerevisiae antibodies and anti-OmpC antibodies are suggestive of fungal or bacterial triggers of granuloma formation. 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. 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. 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. Yet, a high prevalence of human herpesvirus type 8 infection might therefore contribute to the poor prognosis of patients with granulomatous CVID.

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. Macrophages are thought to be one of the first cell types to migrate into affected tissue to clear debris and recruit other immune cells. 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. 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. 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. M2 macrophages are activated through IL-4 and IL-13 and secrete extracellular matrix components promoting tissue remodeling. Inflamed tissue in patients with Crohn’s disease predominantly contain M1 macrophages, and these contribute to the intestinal inflammation by disrupting the epithelial barrier in Crohn’s disease. A similar M1 polarization was seen in

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.

Legend

- multinucleated giant cell
- epithelioid cell
- macrophage
- monocyte
- Th cell
- B cell
alveolar macrophages of patients with sarcoidosis.\(^6^0\) 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 myofibrosis,\(^5^1\) and in fibrotic intestinal lesions of patients with Crohn’s disease.\(^5^2\) These studies suggest an M1 activation predominately 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.\(^5^3,5^4\) 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.\(^6^5\) In contrast to epithelioid cells, these multinucleated cells are capable of phagocytosis and cytokine secretion, especially IL-1, TNF-\(\alpha\) and tumor growth factor-\(\beta\).\(^6^6\)

Our understanding of TNF-\(\alpha\) and its role in granuloma integrity is mostly based on tuberculosis animal models.\(^5^7,5^8\) In the absence of TNF, primary granulomas can still be formed. However, granulomas appeared disorganized.\(^6^7,6^8\) 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-\(\alpha\) also has a major role.\(^6^7\)

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.\(^5^9,6^0\) Furthermore, cultured alveolar macrophages of patients with sarcoidosis spontaneously produce more proinflammatory cytokines, including TNF-\(\alpha\), compared with controls,\(^7^1\) and these higher levels were associated with progressive disease.\(^7^2\) TNF-\(\alpha\) production was also found to be increased in monocytes of patients with CVID.\(^7^3\) The TNF 488A allele leads to higher TNF production and is strongly positively associated with granulomatous CVID.\(^7^4\) 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.\(^7^5\) The intestinal microbiota can also affect the inflammatory environment. Intestinal macrophages of patients with Crohn’s disease produced more proinflammatory cytokines such as TNF-\(\alpha\) after stimulation with commensal bacteria,\(^7^6\) whereas reduced levels of proinflammatory cytokines were reported in response to \(E.\ coli.\(^7^7-7^9\) 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 \textit{in vitro}.\(^8^0-8^2\) Owing to this apparent decreased macrophage function, it has been proposed that Crohn’s disease should also be considered a primary immunodeficiency.\(^8^3,8^4\)

**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.\(^8^5\)

Naïve 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-\(\gamma\) and IL-12 and protect against intracellular pathogens through the production of IFN-\(\gamma\) and the resulting macrophage activation.\(^5^4,8^5\) Uregulation of cytokines promoting Th1 differentiation have been reported in sarcoidosis: IL-2, IL-12, IL-15 and IL-18.\(^8^6\)

Th cell involvement in sarcoidosis is underpinned by the typical CD4...
T-cell lymphopenia in peripheral blood in combination with CD4 T-cell infiltrates at the site of inflammation, such as in bronchoalveolar lavage fluid. 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. CD4 T-cell anergy in these patients is likely due to chronic stimulation and results from reduced availability of G proteins, and reduced nuclear factor-κB capacity of these cells.

Patients with Crohn’s disease show overexpression of IL-12 in intestinal tissue leading to increased production of IFN-γ. 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. The hyperactive state of inflammation in Crohn’s disease is further illustrated by mucosal T-cell proliferation and expansion with resistance to apoptosis. Unlike sarcoidosis and Crohn’s disease, patients with granulomatous CVID have low levels of total T cells and naive CD4 T cells. 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 if 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.

More recently, Th17 have been shown to be disruptive in chronic inflammatory diseases. 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. 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. 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. 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. 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.

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. In contrast, CVID patients have low Th17 cell numbers in their peripheral blood, which is associated with higher numbers of CD21low B cells and lower numbers of memory B cells. The presence of an expanded CD21low B-cell population in CVID patients is associated with higher incidence of non-infectious complications. 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.

Tregs are important to dampen immune responses and thereby maintain a physiological immune homeostasis and self-tolerance. 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. 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. 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.

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. These Tregs inhibited T-cell proliferation, yet several groups confirmed a decreased suppressor function on CD4+ cells. Moreover, Tregs from patients with active sarcoidosis were not able to suppress granuloma formation in an in vitro model, whereas Tregs cells from healthy controls were. 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. The anti-inflammatory function of Tregs is likely to be intact as they have preserved suppressor function, and are able to inhibit effector T-cell responses. 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. 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. 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. 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 autoimmune diseases.

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. Furthermore, several studies showed that numerous B cells surround granulomas in affected tissues from patients with sarcoidosis as well as Crohn’s disease. 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. 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. 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. 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 function in T-cell maturation. 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.\textsuperscript{118,125,126} Despite their reduced numbers, levels of somatic hypermutations in Ig gene transcripts of these cells were increased, suggestive of chronic activation.\textsuperscript{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.\textsuperscript{125,126} Furthermore, the levels of nuclear factor-kB transcription factors in B cells are reduced and potentially affect B-cell responses and proliferation.\textsuperscript{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.\textsuperscript{128,129} In addition, transitional B cells and anergic CD21\textsuperscript{low} B cells were found to be expanded.\textsuperscript{130} Expansions of CD21\textsuperscript{low} B cells are an indication of chronic activation,\textsuperscript{131} as were the observed increased levels of somatic hypermutations in Ig gene transcripts.\textsuperscript{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 hypogammaglobulinemia and all patients have reduced blood plasma cells, which in many patients is accompanied by memory B-cell defects.\textsuperscript{103} Furthermore, in subgroups of patients, expansions of transitional B cells, as well as CD21\textsuperscript{low} B cells, have been identified. Many of these abnormalities have formed the basis of flow cytometry-based classifications.\textsuperscript{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.\textsuperscript{72,103} 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.\textsuperscript{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 regulation and T-cell gut homing.\textsuperscript{146} These abilities could explain the reduced effectiveness of etanercept in Crohn’s disease and sarcoidosis, as well as observed disease complications. Treatment with TNF\alpha blockers also affects the blood B-cell compartment in patients with Crohn’s disease and sarcoidosis.\textsuperscript{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 TNFR1 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.\textsuperscript{148} Abatacept is a recombinant fusion protein of CTLA4 with an immunoglobulin. CTLA-4 inhibits T-cell activation by binding to CD80 on T cells. Abatacept has shown beneficial effects in RA patients,\textsuperscript{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.

**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.\textsuperscript{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.\textsuperscript{28} Despite fibrosis leading to increased morbidity and mortality,\textsuperscript{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.\textsuperscript{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-\alpha and IFN-\gamma).\textsuperscript{134} Methotrexate inhibits the purine metabolism and azathioprine purine synthesis, which both lead to decreased lymphocyte proliferation and cytokine release.\textsuperscript{135} While these therapies are administered to suppress proinflammatory cytokines by inhibiting T-cell responses, these immunosuppressive drugs also affect the B-cell compartment.\textsuperscript{136,137} With the introduction of biological therapies, a third line of treatment has become available, of which TNF\alpha blockers are most notable. The most widely used TNF\alpha blockers are antibodies against TNF\alpha (infliximab and adalimumab), which have proven to be effective in Crohn’s disease and sarcoidosis (Figure 4).\textsuperscript{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 TNF\alpha blockers.\textsuperscript{140} TNF\alpha blockers infliximab and etanercept have proven to be beneficial in some patients with granulomatous CVID.\textsuperscript{52,141} Etanercept is a recombinant TNF\alpha receptor fused to an Ig constant region and is often used to treat RA.\textsuperscript{142} Importantly, etanercept is not effective in sarcoidosis and Crohn’s disease, and can even lead to increased disease activity in these disorders.\textsuperscript{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-\alpha; (2) etanercept has low affinity to transmembrane TNF;\textsuperscript{145} (3) etanercept binds to both TNF-\alpha and lymphotoxin-\alpha, a cytokine that is crucial for secondary lymphoid organ development, IgA regulation and T-cell gut homing.\textsuperscript{146} These abilities could explain the reduced effectiveness of etanercept in Crohn’s disease and sarcoidosis, as well as observed disease complications. Treatment with TNF\alpha blockers also affects the blood B-cell compartment in patients with Crohn’s disease and sarcoidosis.\textsuperscript{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 TNFR2 that is expressed on B cells.

Clinical & Translational Immunology
Targeting of Th17 responses have also been studied. However, blocking IL-17 with secukinumab was ineffective in patients with Crohn’s disease, whereas treatment with brodalumab, an anti-IL-17 receptor monoclonal antibody, even resulted in exacerbation of Crohn’s disease. Ustekinumab, a monoclonal antibody against both IL-12 and IL-23, resulted in a clinical response in patients with refractory Crohn’s disease and is currently implemented in patients who are resistant to TNFα blockers. However, ustekinumab did not show therapeutic efficacy in sarcoidosis patients. Patients with Crohn’s disease do show a good response to treatment with vedolizumab, a humanized monoclonal antibody that binds to integrin α4β7. As α4β7 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. Rituximab is a humanized anti-CD20 antibody that depletes all naive and memory B cells. The efficacy of rituximab in sarcoidosis is still unclear: several case reports show proven effectiveness; however, in one small prospective study with 10 patients, only 5 of them showed a marginal (> 5%) improvement of respiratory function. 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. 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. 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. 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 that manifests with early-onset inflammatory bowel disease.

Interestingly, the key in therapy might lie in improving T-cell function by targeting of the inhibitory receptor programmed death-1 (PD-1). In patients with sarcoidosis, PD-1 expression is increased on T cells in granulomatous tissue, and the number of PD-1-expressing Th cells in blood are increased. 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. 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. Still, a cautious approach is warranted as sarcoidosis-induced disease also has been reported by the use of pembrolizumab in a patient with sarcoma. 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. Moreover, ICOSL gene polymorphisms are associated with Crohn’s disease, and increased...
ICOS expression was reported on Tregs in patients with sarcoidosis. A monoclonal antibody targeting ICOSL, AMG-557, has been developed and is currently undergoing the first trial in systemic lupus erythematosus. 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.

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. Especially autoreactive B cells are dependent on high BAFF levels. As patients with active sarcoidosis and CVID display increased BAFF levels, it likely contributes to disease pathogenesis. Thus, belimumab might be more effective than rituximab through stronger effects on pathogenic B cells.

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. For example, only 60% of patients with Crohn’s disease achieved short-term disease control. 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. Treatment monitoring through immunological tests are another option. Specifically, quantification of serum soluble IL-2R levels is routinely used for patients with sarcoidosis, because it correlates with pulmonary function tests and with local disease activity as visualized by fluorine-18 fluorodeoxyglucose positron emission tomography scan.

Quantification of serum BAFF levels could be a good biomarker as it is elevated in chronic active sarcoidosis. 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. Furthermore, successful infliximab therapy in Crohn’s disease resulted in a normalization of IgM memory B-cell numbers. 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.

ACKNOWLEDGEMENTS
We thank Dr LSJ Kamphuis and Dr KH Lam for their support with immunohistochemistry of biopsy samples.

Clinical & Translational Immunology

1 Medzhitov R. Origin and physiological roles of inflammation. Nature 2008; 454: 428–435.
2 Doria A, Zen M, Bettio S, Gatto M, Bassi N, Nalotto L et al. Autoinflammation and autoimmunity: bridging the divide. Autoimmun Rev 2012; 12: 22–30.
3 Mukhopadhyay S, Farver CF, Vazdar LT, Dempsey OJ, Popper HH, Mani H et al. Causes of pulmonary granulomas: a retrospective study of 500 cases from seven countries. J Clin Pathol 2012; 65: 51–57.
4 Williams GT, Williams WJ. Granulomatous inflammation—a review. J Clin Pathol 1983; 36: 723–733.
5 Orme IM, Basaraba RJ. The formation of the granuloma in tuberculosis infection. Semin Immunol 2014; 26: 601–609.
6 Woodard BH, Rosenberg SI, Farnham R, Adams DO. Incidence and nature of primary granulomatous inflammation in surgically removed material. Am J Surg Pathol 1982; 6: 119–129.
7 Levine S, Smith VV, Malone M, Sebire NJ. Histopathological features of chronic granulomatous disease (CGD) in childhood. Histopathology 2005; 47: 508–516.
8 Rosenman MD. Chronic beryllium disease: a hypersensitivity disorder. Appl Occup Environ Hyg 2001; 16: 615–618.
9 Mukhopadhyay S, Gal AA. Granulomatous lung disease: an approach to the differential diagnosis. Arch Pathol Lab Med 2010; 134: 667–670.
10 Valeyre D, Prasse A, Nunes H, Uzunhan Y, Brillet PY, Muller-Quernheim J. Sarcoidosis. Lancet 2014; 383: 1159–1167.
11 Baumgart DC, Sandborn WJ. Crohn’s disease. Lancet 2012; 380: 1590–1605.
12 Ardenæs O, Cunningham-Rundles C. Granulomatous disease in common variable immunodeficiency. Clin Immunol 2009; 133: 198–207.
13 Tai SW, Baeten DL. Recent advances in the treatment of immune-mediated inflammatory diseases. Methods Mol Biol 2016; 1371: 143–155.
14 Iannuzzi MC, Rybicki BA, Steinstein AS. Sarcoidosis. N Engl J Med 2007; 357: 2153–2165.
15 Hungthongke GW, Costabel U, Ando M, Baughman R, Cordier JF, du Bois R et al. Statement on sarcoidosis. Joint Statement of the American Thoracic Society (ATS), the European Respiratory Society (ERS) and the World Association of Sarcoidosis and Other Granulomatous Disorders (WASOG) adopted by the ATS Board of Directors and by the ERS Executive Committee, February 1999. Am J Respir Crit Care Med 1999; 160: 736–755.
16 Scadding JG. Prognosis of intrathoracic sarcoidosis in England. A review of 136 cases after five years’ observation. BMJ 1961; 2: 1165–1172.
17 Treglia G, Taralli S, Giordano A. Emerging role of whole-body [18F]-fluorodeoxyglucose positron emission tomography as a marker of disease activity in patients with sarcoidosis: a systematic review. Sarcoidosis Vascul Dis Lung 2011; 28: 87–94.
18 Nijhuis AC, Feilrath JM, Muider H, Janssen R, van den Bosch JM, van Velsen-Blad H. Serum soluble interleukin-2 receptor measurement in patients with sarcoidosis: a clinical evaluation. Chest 2003; 124: 186–195.
19 Drent M, Cremer JP, Jansen TL, Baughman RP. Practical emergence and experience-based recommendations for use of TNF-α inhibitors in sarcoidosis. Sarcoidosis Vascul Dis Lung 2014; 31: 91–107.
20 Patterson KC, Hogarth K, Husain AN, Sperling AI, Niewold TB. The clinical and immunologic features of pulmonary fibrosis in sarcoidosis. Transl Res 2012; 160: 321–331.
21 Burisch J, Munkholm P. The epidemiology of inflammatory bowel disease. Scand J Gastroenterol 2015; 50: 942–951.
Immunopathogenesis of granulomas

WM Timmermans et al

22 Geremia A, Biancheri P, Allan P, Corazza GR, Di Sabatino A. Innate and adaptive immunity in inflammatory bowel disease. Autoimmun Rev 2014; 13: 3–10.

23 Heresbach D, Alexandre JL, Branger B, Bretagne JF, Coudert E, Dabadie A et al. Frequency and significance of granulomas in a cohort of incident cases of Crohn's disease. Gut 2006; 54: 215–222.

24 Feaksin RM. Ulcerative colitis or Crohn's disease? Pitfalls and problems. Histoopathology 2014; 64: 317–335.

25 Sendid B, Colombel JF, Jacquotin PM, Faillie C, Fruit J, Cortot A et al. Specific antibody response to oligomannan epitopes in Crohn's disease. Clin Diag Lab Immunol 1996; 3: 219–226.

26 Mei L, Targan SR, Landers CJ, Dutridge D, Ippoliti A, Vasiliauskas EA. IL-23 accompanies the inflammation of mycobacterial and propionibacterial DNA in lymph nodes of Japanese and European patients with Crohn's disease. Gastroenterology 2005; 129: 1079–1085.

27 Colombe JF, Sandborn WJ, Reinish W, Mantzaris GJ, Kornbluth A, Rachmilewitz D et al. Inflammasome, azathioprine, or combination therapy for Crohn's disease. N Engl J Med 2010; 362: 1383–1395.

28 Gathmann B, Mahlaoui N, Ceredihi, Gerard L, Oksenhendler E, Warnatz K et al. Clinical picture and treatment of 2212 patients with common variable immunodeficiency. J Allergy Clin Immunol 2014; 134: 116–126.

29 Conley ME, Notarangelo LD, Eizorni A. Diagnostic criteria for primary immunodeficiencies. Representing PAGID (Pan-American Group for Immunodeficiency) and ESID (European Society for Immunodeficiencies). Clin Immunol 1999; 93: 190–197.

30 Resnick ES, Mosher EL, Godbold JH, Cunningham-Rundles C. Morbidity and mortality in common variable immune deficiency caused by mutations in the PIK3R1 gene. J Pediatr 2014; 165: S127–134.

31 Resnick ES, Moshier EL, Godbold JH, Cunningham-Rundles C. Frequency and significance of granulomas in a cohort of incident cases of Crohn's disease. Am J Gastroenterol 2002; 97: 2011–2012.

32 van Zelm MC, Smit J, Adams B, Mascart F, Schandene L, Janssen F et al. CD81 gene defect in humans disrupts CD9 complex formation and leads to antibody deficiency. J Clin Invest 2010; 120: 1269–1274.

33 Warnatz K, Salzer U, Rizzi M, Fischer B, Guttenberger S, Bohn J et al. B-cell activating factor receptor deficiency is associated with an adult-onset antibody deficiency syndrome. Proc Natl Acad Sci USA 2009; 106: 13945–13950.

34 Angulo I, Vadás G, Garcon F, Banham-Hall E, Plagnol V, Leary TR et al. Phosphoinositide 3-kinase delta gene mutation predisposes to respiratory infection and airway damage. Science 2013; 342: 866–870.

35 Deau MC, Heurtier L, Frange P, Suarez F, Bole-Foyer C, Nitschke P et al. A human immunodeficiency caused by mutations in the PIK3R1 gene. J Clin Immunol 2014; 124: 3923–3928.

36 Fliegauf M, Bryant VL, Frede N, Oksenfenner E, Warnatz K et al. Haploinsufficiency of the NF-kappaB1 subunit p50 in common variable immunodeficiency. Am J Hum Genet 2015; 97: 389–403.

37 Kuehn HS, Ouyang W, Lo B, Deenick EK, Niemela JE, Avery DT et al. Immune dysregulation in humans with heterozygous germline mutations in CTLA4. Science 2014; 345: 1623–1627.

38 Bouzid D, Mouhod B, Brillet PY, Kambouchner M, Ducrop JJ, Cottin V et al. Granulomatosis-associated common variable immunodeficiency disorder: a case-control study versus sarcoidosis. Eur Respir J 2013; 41: 115–122.

39 Mannon PJ, Fuss UJ, Dill S, Friend J, Groden C, Hromng R et al. Excess IL-12 but not IL-10 expression is associated with sarcoidosis in common variable immunodeficiency. Gastroenterology 2006; 131: 748–756.

40 Bates CA, Ellison MC, Lynch DA, Cool CD, Brown KK, Routes JM. Alveolar macrophage cell fusion or proliferation with non-division? Am J Pathol 2000; 157: 706–710.

41 Okamoto H, Mizuno K, Horio T. Monocyte-derived multinucleated giant cells and sarcoidosis. J Dermatol Sci 2003; 31: 119–128.

42 Heinrichs H, Zettel G, Goldmann T, Tschernig T, Vollmer E, Pabst R et al. Alveolar macrophage cell fusion or proliferation with non-division? Am J Pathol 2000; 157: 706–710.

43 Aukrust P, Uien E, Kristoffersen AK, Muller F, Haug CJ, Espesvik T et al. Persistent activation of the tumour necrosis factor system in a subgroup of patients with common variable immunodeficiency—perhaps immunologic and clinical consequences. Blood 1996; 87: 674–681.

44 Mullighan CG, Fanning GC, Chapel HM, Welsh KI. TNF and lymphotoxin-alpha–beta polymorphisms associated with common variable immunodeficiency: role in the pathogenesis of granulomatous disease. J Immunol 1997; 159: 6236–6241.

45 Mendoza JL, Lana R, Diaz-Rubio M. Mycobacterium avium subspecies paratuberculosis and its relationship with Crohn's disease. World J Gastroenterol 2009; 15: 417–422.

46 Boursiquot JN, Gerard L, Malphettes M, Fieschi C, Gaicher L, Boutboul D et al. Granulomatous disease in Crohn's disease: immunohistological analysis of clinical characteristics and treatment efficacy in a cohort of 59 patients. J Clin Immunol 2013; 33: 84–95.

47 Wheat WH, Cool CD, Morimoto Y, Rai PR, Kirkpatrick CH, Lindenbaum BA et al. Possible role of human herpesvirus 8 in the lymphoproliferative disorders in common variable immunodeficiency. J Exp Med 2004; 199: 479–484.

48 Arango Duque G, Descoteaux A. Macrophage cytokines: involvement in immunity and infectious diseases. Front Immunol 2014; 5: 491.

49 Murray PJ, Allen JE, Biswas SK, Fisher EA, Gilroy DW, Goedert S et al. Macrophage activation and polarization: nomenclature and experimental guidelines. Immunol 2014; 41: 14–20.

50 Wynn TA, Chawla A, Pollard JW. Macrophage biology in development, homeostasis and disease. Nature 2013; 496: 445–455.

51 Mosser DM, Edwards JP. Exploring the full spectrum of macrophage activation. Nat Rev Immunol 2008; 8: 958–969.
60 Darfeuille-Michaud A, Adherent-invasive Escherichia coli: a putative new E. coli pathotype associated with Crohn's disease. *Int J Med Microbiol* 2002; 292: 185–193.

61 Ryan P, Kelly RG, Lee G, Collins JK, O'Sullivan GC, O'Connell J. Ryan et al. Revisiting Crohn's disease as a primary immunodecit of macrophages. *J Exp Med* 2006; 206: 1839–1843.

62 Gambineri E, Perroni L, Passerini L, Bianchi L, Doglioni C, Meschi F. Gambineri et al. Resolution of pulmonary sarcoidosis. *Am J Respir Crit Care Med* 2010; 181: 1730–1738.

63 Ryan P, Kelly RG, Lee G, Collins JK, O'Sullivan GC, O'Connell J. Ryan et al. Revisiting Crohn's disease as a primary immunodecit of macrophages. *J Exp Med* 2006; 206: 1839–1843.

64 Marka S, Kanai T, Osabuishi R, Tomita T, Sawada T et al. CD20-Specific T Cells and Tumoral Intestinal lamina propria as regulatory cells. *J Immunol* 2004; 173: 3119–3130.

65 Mail J, Loddenkemper C, Mundt P, Berg E, Giese T, Stallmach A et al. Peripheral and intestinal regulatory CD4+CD25(high) T cells in inflammatory bowel disease. *J Immunol* 2010; 184: 4495–4503.

66 Mignon P, Cattaneo M, Savoldo B, Armuzzi L, Golino P, Rinaldi M. Mignon et al. Peripheral CD4+ CD25(high) regulatory T cells in Crohn's disease. *J Immunol* 2009; 183: 954–962.

67 Tafint C, Miyata M, Nochy D, Valeyre D, Naccache JM, Altare F et al. FoTx3+ regulatory T cells suppress early stages of granuloma formation but have little impact on sarcoidosis lesions. *Am J Pathol* 2009; 174: 497–508.

68 Nakata S, Kanai T, Oshima S, Urashihara K, Tsubota T, Sawada T et al. CD4+CD25+FOXP3+ Regulatory T Cells in Patients with Sarcoidosis. *Am J Respir Crit Care Med* 2011; 183: 185–192.

69 Pozzi E, Di Sabatino A, Rosado MM, Cazzola P, Morera R, Corazza GR et al. Pozzi et al. Immunopathogenesis of granulomas. *WM Timmermans et al.*

70 Imboden JB, Spierer MA. Peripheral anergy and local immune hyperactivation in Crohn's disease. *J Clin Immunol* 2010; 30: 20–31.

71 Leong S, Liu J, Fu X, Fan C, Guo T, Zhang L. The cytokine milieu in the interplay of pathogenic Th17 cells and regulatory T cells in autoimmune disease. *Cell Mol Immunol* 2010; 7: 182–189.

72 Chen ES, Moller DR. Sarcoidosis—scientific progress and clinical challenges. *Nat Rev Rheumatol* 2011; 7: 457–467.

73 Moller DR, Forman JD, Liu MC, Noble PW, Greenlee BM, Vyas P et al. Enhanced expression of IL-12 associated with Th1 cytokine profiles in active pulmonary sarcoidosis. *J Immunol* 1996; 156: 4952–4960.

74 Inui N, Chida K, Suda T, Nakamura H. Th1/Th2 and Th1/Th2 profiles in peripheral blood and bronchoalveolar lavage fluid cells in pulmonary sarcoidosis. *J Allergy Clin Immunol* 2001; 107: 337–344.

75 Bianco A, Spiteri MA. Peripheral anergy and local immune hyperactivation in Crohn's disease: a paradox of a feather. *Exp Clin Immunogenet* 2003; 20: 1–3.

76 Nemno G, Prigent AF, Aloui R, Charpin G, Gormand F, Gallet H et al. Impaired G-proteins and cyclic nucleotide phosphodiesterease activity in T-lymphocytes from patients with sarcoidosis. *Eur J Clin Invest* 1993; 23: 18–27.

77 Lee NS, Barber L, Kadowa A, Childs SJ, Lickon MA et al. Low levels of NF-kappaBp65 mark anergic CD4+ T cells and correlate with disease severity in sarcoidosis. *Clin Vaccine Immunol* 2011; 18: 223–234.

78 Monte Leone G, Biancone L, Marasco R, Morone G, Marasco O, Luzzo F et al. Interleukin 12 is expressed and actively released by Crohn's disease intestinal lamina propria mononuclear cells. *Gastroenterology* 1997; 112: 1169–1178.

79 Garcia de Tena J, Manzano L, Leal JC, San Antonio E, Sualeva V, Alvarez-Mon M. Active Crohn's disease patients show a distinctive expansion of circulating memory CD4+CD62LBlind T cells. *J Clin Immunol* 2004; 24: 185–196.

80 de Souza HS, Fiocchi C. Immunopathogenesis of IBD: current state of the art. *Nat Rev Gastroenterol Hepatol* 2016; 13: 13–27.

81 Cosmi S, Liotta F, Maggi E, Romagnani S, Anzaniutto F. Th17 and non-classic Th1 cells in chronic disorders: two sides of the same coin. *Int Arch Allergy Immunol* 2014; 164: 171–177.

82 Genovese MC, Durez P, Richards HB, Supronik J, Dokoupilova E, Mazurov V et al. Efficacy and safety of safety-switching in patients with rheumatoid arthritis: a phase II, dose-finding, double-blind, randomised, placebo-controlled study. *Ann Rheum Dis* 2013; 72: 863–869.

83 Steinman L. A brief history of Th17, the first major revision in the Th1(Th2) hypothesis of T cell-mediated tissue damage. *Nat Med* 2007; 13: 139–145.

84 Anzaniutto F, Cosmi S, Liotta F, Maggi E, Liotta F, Mazurov V et al. Phenotypic and functional features of human Th17 cells. *J Exp Med* 2007; 204: 1849–1861.

85 Harbour SN, Maynard CL, Zindl CL, Schoeb TR, Weaver CT. Th17 cells give rise to Th10 cells that react for the pathogenesis of colitis. *Proc Natl Acad Sci USA* 2015; 112: 7061–7066.

86 Gagliani N, Amexzas Vesely MC, Iseppon A, Brockmann L, Hu X, Palm NW et al. Th17 cells transdifferentiate into regulatory T cells during resolution of inflammation. *Nature* 2016; 533: 221–222.

87 Ten Berge B, Paats MS, Bergen IM, van den Blink B, Hoogsteden HC, Lambrecht BN et al. Increased IL-17A expression in granulomas and in circulating memory T cells in sarcoidosis. *Rheumatology* 2012; 51: 37–46.

88 Barbosa RR, Silva SP, Silva SL, Melo AC, Pedro E, Barbosa MP et al. Primary B-cell deficiencies reveal a link between human IL-17-producing CD4+ Th17 cell homeostasis and B-cell differentiation. *PLoS ONE* 2011; 6: e22848.

89 Wehr C, Kivioja T, Schmitt C, Ferry B, Witlet E, Eren E et al. The EUROclass trial: defining subgroups in common variable immunodeficiency. *Blood* 2008; 111: 77–85.

90 Sakaguchi S, Miyara M, Costantino CM, Hafler DA. FODP3+ regulatory T cells in the human immune system. *Nat Rev Immunol* 2010; 10: 490–500.

91 Sakaguchi S, Sakaguchi N, Asano A, Itoh M, Toda M. Immunologic self-tolerance maintained by active deletion of cells expressing U-2 receptor alpha-chains (CD25). Breakdown of a single mechanism of self-tolerance causes various autoimmune diseases. *J Immunol* 1995; 155: 1151–1164.

92 Gambineri E, Perroni L, Passerini L, Bianchi L, Dogliani C, Meschi F et al. Clinical and molecular profiling of new series of patients with immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome: inconsistent correlation between forkhead box protein 3 expression and disease severity. *J Allergy Clin Immunol* 2008; 122: 1109–1105.e2.

93 Myhal A, Amoury T, Parizot C, Saudous C, Donghun K. Trad S et al. The immune paradox of sarcoidosis and regulatory T cells. *J Exp Med* 2006; 203: 359–370.

94 Oswald-Richter KA, Richmond BW, Braun NA, Isom J, Abraham S, Taylor TR et al. Reversal of global CD4+ subset dysfunction is associated with spontaneous clinical improvement in different clinical subgroups of patients with immune dysregulation, polyendocrinopathy, enteropathy, X-linked syndrome. *J Immunol* 2009; 183: 1541–1549.

95 Rappi G, Pabst S, Riemann D, Schmidt A, Wickenhauser C, Schutte W et al. Regulatory T cells with reduced effector capacities are extensively amplified in pulmonary sarcoid lesions and sustain granuloma formation. *Clin Immunol* 2011; 140: 71–83.
Clinical & Translational Immunology

Immuneopathogenesis of granulomas
WM Timmersma et al.

140 Keane J, Gershon S, Wise RP, Mittlep-Levens E, Kasznica J, Schwiederman WD et al. Tuberculosis associated with inflammasome, a tumor necrosis factor alpha-neutralizing agent. N Engl J Med 2001; 345: 1098–1104.

141 Franxman TJ, Howe LE, Baker JR Jr. Influenza for treatment of granulomatous disorders. In patients with common variable immunodeficiency. J Clin Immunol 2014; 34: 820–827.

142 Moreland LW, Baumgartner SW, Schiffr MH, Tindall EA, Fleischmann RM, Weaver AL et al. Treatment of rheumatoid arthritis with a recombinant human tumor necrosis factor receptor (p75 TNF receptor) fusion protein. N Engl J Med 1997; 337: 141–147.

143 Sandborn WJ, Hanauer SB, Katz S, Safdi M, Wolf DG, Raab R et al. Etanercept for active Crohn’s disease: a randomized, double-blind, placebo-controlled trial. Gastroenterology 2001; 121: 1088–1094.

144 Louie GH, Chikwanda P, Ward DJ. Relapse of sarcoidosis upon treatment with etanercept. Ann Rheum Dis 2008; 67: 896–898.

145 Ehlers S. Tumor necrosis factor and its blockade in granulomatous infections: differential modes of action of inflammasome and etanercept? Curr Inflam Dis Rep 2005; 41 (Suppl 3): S199–S203.

146 Ruddle NH. Lymphotixin and TNF: how it all began—a tribute to the travelers. Cytokine & growth factor reviews 2014; 25: 83–89.

147 Di Sabatino A, Rosado MM, Cazzola P, Biancheri P, Tinozzi FP, Laer MA et al. Splenic function and IgM-memory B cells in Crohn’s disease patients treated with infliximab. Int J Clin Pharmacol Ther 2008; 14: 591–596.

148 Sandborn WJ, Colombel JF, Sands BE, Rutgeerts P, Hanauer S, Colombel JF, Sands BE et al. Abatacept for Crohn’s disease and ulcerative colitis. Gastroenterology 2012; 143: 62–69 e4.

149 Kremer JM, Westhovens R, Leon M, Di Giorgio E, Alten R, Steinfeld S et al. Ignatenko S, Skrumsager BK, Mouritzen U. Safety PK and PD of recombinant anti-T-cell-co-stimulator ligand (ICOSL) blockade leads to selective inhibition of anti-KLH IgG responses in subjects with systemic lupus erythematosus. Lupus Sci Med 2016; 3: e00146.

150 Vincent FB, Morandi EF, Schneider P, Mackay F. The BAFF/APRIL system in sLE pathogenesis. Nat Rev Rheumatol 2014; 10: 365–373.

151 Mackay F, Woodcock SA, Lawton P, Ambrose C, Baebersch, M Schneider P et al. Mice transgenic for BAFF deficient lymphopoiesis along with autoimmune manifestations. J Exp Med 1999; 190: 1677–1710.

152 Baert F, Noman M, Vermeire S, Van Assche G, DH Barone A et al. Influence of immunogenicity on the long-term efficacy of infliximab in Crohn’s disease. N Engl J Med 2003; 348: 601–608.

153 Keijser RG, Verzijlberg JF, van Diepen DM, van den Bosch JM, Gut SC et al. 1F–FCDG PET in sarcoidosis: an observational study in 12 patients treated with infliximab. Sarcoidosis Vasc Diffuse Lung Dis 2008; 25: 143–149.

154 Vorseelsa A, van Moorel CH, Zaran P, Ruven JH, Claessen AM, van Vetel-Bh et al. ACE and sIL-2R correlate with lung function improvement in sarcoidosis during methotrexate therapy. Respir Med 2015; 109: 279–285.

155 Verwoerd A, Hijdra D, Vorseelsa A, Cormeel HA, van Moorel CH, Gut SC et al. Infliximab therapy balances regulatory T cells, TNR2 expression and sTNR2 in sarcoidosis. Clin Exp Immunol 2016; 185: 263–270.

156 Li Z, Vermeire S, Bullens D, Ferrante M, Van Steen K, Noman M et al. Restoration of Foxp3+ regulatory T-cell subsets and Foxp3–1 type-1 regulatory T cells in inflammatory bowel diseases during anti-tumor necrosis factor therapy. Inflamm Bowel Dis 2016; 22: 1215–1224.

157 Kakazu T, Hara J, Matsumoto T, Nakamura S, Oshifumi N, Arakawa T et al. T helper 1 cell predominance in granulomas of Crohn’s disease. Am J Gastroenterol 2009; 104: 2149–2155.

158 Senju M, Huata S, Lowter J, Weijl DP. Flow cytometric analysis of peripheral blood lymphocytes in ulcerative colitis and Crohn’s disease. Gut 1991; 32: 779–783.

159 Naser SA, Romero C, Urbina P, Naser N, Valentine J. Cellular infiltration and cytokine expression correlate with fistulizing state in Crohn’s disease. Clin Vaccine Immunol 2013; 18: 1416–1419.

160 Faccio M, Cabrellie A, Teramo A, Oliveri V, Groato M, Teolato S et al. Sarcoidosis is a Th1/Th17 multisystem disorder. Thorax 2011; 66: 144–150.

161 Rezaei N, Aghamohammadi A, Kardar GA, Mounizadeh M, Pourpaz Z. T helper-1 and 2 cytokine assay in patients with common variable immunodeficiency. J Immunol Res 2016; 2016: 489–493.

162 Paronchi P, Romagnani P, Annunziato F, Sampognaro S, Bechi A, Giannarini L et al. Type 1 T-helper cell predominance and interleukin-12 expression in the gut of patients with Crohn’s disease. J Am Pathol 1997; 150: 823–832.

163 Karttunen R, Breese EJ, Walker-Smith JA, McDonald TT. Decreased mucosal interleukin-4 (IL-4) production in gut inflammation. J Clin Pathol 1994; 47: 1015–1018.

164 Faccio M, Cabrellie A, Teramo A, Oliveri V, Groato M, Teolato S et al. Sarcoidosis is a Th1/Th17 multisystem disorder. Thorax 2011; 66: 144–150.

165 Rezaei N, Aghamohammadi A, Kardar GA, Mounizadeh M, Pourpaz Z. T helper-1 and 2 cytokine assay in patients with common variable immunodeficiency. J Immunol Res 2016; 2016: 489–493.

166 Swaai NJ, Salloum R, Gandhi S, Aglee ML, Sawadzki R, Badaracco M et al. Significant CD4, CD8, and CD19 lymphopenia in peripheral blood of sarcoidosis patients correlates with severe disease manifestations. PLoS ONE 2010; 5: e00988.

167 Balbi E, Bruchel A, Dupieux C, Karslijus N, Sletsgaard B, Pel P et al. Th17 polarization in sarcoidosis, clinical features, diagnosis, treatment, and management. Infect Drug Resist 2014; 7: 183–197.

168 Petersen HJ, Smith AM. The role of the innate immune system in granulomatous disorders. Front Immunol 2013; 4: 120.

169 Fais S, Pallone F. Inability of normal human intestinal macrophages to form multinucleated giant cells in response to cytokines. Gut 1995; 37: 798–801.

170 Vas AC, Wildenberg ME, Duijvestein M, Verhaar AP, de Hertogh G. Anti-tumor necrosis factor-alpha inhibitors induce regulatory macrophage in an Fc receptor-dependent manner. J Immunol 2010; 14: 270–279.

171 Neurath MF, Travis SP. Mucosal healing in inflammatory bowel diseases: a systematic review. Gut 2012; 61: 1619–1635.

This work is licensed under a Creative Commons Attribution-NonCommercial-ShareAlike 4.0 International License. The images or other third party material in this article are included in the Creative Commons License, unless indicated otherwise in the credit line; if the material is not included under the Creative Commons license, users will need to obtain permission from the license holder to reproduce the material. To view a copy of this license, visit http://creativecommons.org/licenses/by-nc-sa/4.0/.

© The Author(s) 2016