Pathogenesis of ocular tuberculosis: new observations and future directions

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Keywords: tuberculosis; mycobacterium; ocular; uveitis; pathogenesis; autoimmunity

Article highlights

- Pathomechanisms in ocular TB are likely to be multi-factorial
- *Mycobacterium tuberculosis* may be directly or indirectly implicated
- Indirect mechanisms include autoimmune response in eye and remote immune priming
- Diagnosis and treatment should target the predominant mechanism

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Abstract

Ocular tuberculosis (OTB) encompasses all forms of intra- and extra-ocular inflammation associated with *Mycobacterium tuberculosis* (*Mtb*) infection. However, the organism is rarely found in ocular fluid samples of diseased eyes, rendering the pathomechanisms of the disease unclear. This confounds clinical decision-making in diagnosis and treatment of OTB. Here, we critically review existing human and animal data related to ocular inflammation and TB pathogenesis to unravel likely pathomechanisms of OTB. Broadly there appear to be two fundamental mechanisms that may underlie the development of TB-associated ocular inflammation: a. inflammatory response to live/ replicating *Mtb* in the eye, and b. immune mediated ocular inflammation induced by non-viable *Mtb* or its components in the eye. This distinction is significant as in direct *Mtb*-driven mechanisms, diagnosis and treatment would be aimed at detection of *Mtb*-infection and its elimination; while indirect mechanisms would primarily require anti-inflammatory therapy with adjunctive anti-TB therapy. Further, we discuss how that most clinical phenotypes of OTB likely represent a combination of both mechanisms, with one being predominant than the other.

1 Introduction

Ocular tuberculosis (OTB) is a broad term, used for intraocular inflammation associated with evidence of *Mycobacterium tuberculosis* (*Mtb*) infection in the eye or elsewhere in the body, along with exclusion of all possible non-TB entities [1-3]. *Mtb*-associated intraocular inflammation can result in moderate to severe visual impairment in at least 40% affected eyes [4] and is being increasingly reported from both TB-endemic and non-endemic countries [5,6]. The disease can affect nearly every tissue in the eye and therefore has varied clinical presentations [1-3]. These clinical presentations have been listed in Table 1 and illustrated in Figure 1. Some of the clinical signs are highly predictive of OTB, at least in TB-endemic countries, while others are non-specific and can be found in other forms of infectious and non-infectious uveitis (intraocular inflammation) as well [1-3,7,8]. The definitive diagnosis of OTB is based on demonstration of *Mtb* or *Mtb*-DNA with or without the presence of granulomatous inflammation in uveal and retinal tissues [9]. Since these ocular tissues are generally not accessible for sampling, etiological diagnosis of TB is often based on presence of characteristic ocular signs, ancillary evidence of systemic TB infection, and exclusion of non-TB entities [1-3]. Treatment includes anti-TB therapy (ATT) with concomitant corticosteroid therapy in varying doses and routes. Such treatment has been shown to result in resolution of intraocular inflammation and prevent future recurrences in nearly 84% cases [10]. In the past two decades, there have been rapid advances in understanding the diagnosis and treatment of OTB based on ocular fluid analysis by polymerase chain reaction (PCR), ocular imaging and response to ATT [11-13].

2. Current understanding of pathogenesis of ocular TB

In comparison to the clinical insights, attempts at understanding the pathogenesis of OTB have been significantly more challenging. It has generally been assumed that hematogenous dissemination of *Mtb* to the eye induces intraocular inflammation even though the mechanism of dissemination has been difficult to prove [14]. However,

microbiological or molecular evidence of *Mtb* is rarely found in ocular fluid samples [3]. The diagnostic efficacy of conventional polymerase chain reaction (PCR) for *Mtb* is as low as 37.7% in different forms of granulomatous uveitis [15]. Despite several modifications in molecular diagnostic techniques over the years, the use of *Mtb* PCR in routine practice has remained low [16]. In general, nucleic acid amplification tests are yet to receive complete endorsement for diagnosis of extrapulmonary TB (EPTB) [17]. The rarity of direct evidence of *Mtb* in clinical samples from OTB patients has supported suggestions from experimental and clinical studies that OTB represents a hypersensitivity response to *Mtb* antigens [18-19]. A third possibility remains that the ocular inflammation results from distal immune cell priming of T-cells that cross-react between *Mtb* and ocular antigens, thereby resulting in ocular inflammation even in the absence of bacterial products in the eye [20]. It is possible that there are yet other putative mechanisms or that the true sequence of events in OTB shifts between these mechanisms, in different clinical presentations of the disease. However, there have been relatively limited attempts to explore the merits of each of these possibilities, based on published literature. Developing this knowledge is significant, since the study design and interpretation of outcomes of clinical trials for OTB will remain questionable without a clear understanding of underlying pathogenesis. In this review, we collate the spectrum of human and animal data related to OTB, to develop a unified hypothesis of pathogenesis. The strengths and weaknesses of available data will be interpreted in the context of clinical observations in patients. Finally, the impact of our proposed hypothesis on the diagnosis and management of OTB, and possible strategies for future studies, are discussed.

3. Direct infection-driven pathogenesis of ocular TB

The infectious mechanism of OTB pathogenesis is supported by demonstration of hematogeneous dissemination of *Mtb* to the eye; and/or by histological demonstration of granulomatous inflammation and acid-fast bacilli, in diseased eyes or animal models. Such criteria were also applied to pathogenesis of other forms of extrapulmonary TB (EPTB) [21].

In this section, we will review how OTB represents an extrapulmonary manifestation of TB, highlighting animal data on mycobacterial dissemination to the eye and histological evidence of granulomatous inflammation in human and animal eyes infected with *Mtb*.

3.1 Ocular TB as a form of extrapulmonary TB: host and bacterial factors

OTB is not included in most epidemiological studies on EPTB and has generally been studied in isolation. In general, the prevalence of OTB in uveitis clinics is available from retrospective studies from around the world [22-25]. This ranged from 32.4% in Myanmar to 0.4% in one study from mid-Atlantic United States (Table 2). It is obvious that the prevalence of OTB is directly related to TB-endemicity of the region [26]. However, it is possible that the reported prevalence is also related to evolving diagnostic criteria and changes in diagnostic techniques for OTB. For example, one centre in north India reported a prevalence of 10.1% of OTB in 2004 [27], and 22.9% in 2016 [22].

Despite an apparent association of the prevalence of OTB with TB-endemicity of the region, co-existence of OTB and pulmonary TB (PTB), in individual patients, is relatively uncommon. An early study covering the period of 1940-1966 at a TB sanatorium in USA found 154 cases (1.4%) of ocular TB among 10,524 cases of active pulmonary TB (PTB) [28]. Later, a study from south India found similar prevalence (1.39%) of ocular TB among 1005 cases of PTB [29]. In both series, choroiditis was the most common clinical presentation of OTB, and retinal vasculitis was rarely [28], or not seen [29]. The prevalence of OTB was slightly higher (~10%) when only patients with EPTB were analyzed [30]. Conversely, in patients with OTB, a recent multi-centric study found that 76.7% (604/787) did not have any past history of PTB/EPTB. Only 26.9% had chest X-ray and 68.6% of those tested had computerized tomography features consistent with inactive or healed pulmonary tuberculosis [31]. Together, these data suggest that host and/or bacterial factors responsible for development of OTB are probably different from those of pulmonary TB (PTB).

Apart from the association with PTB, the other host factors that have been studied for OTB are age, gender, ethnicity and HIV status. In the multinational cohort mentioned above [31], the mean age of OTB patients was 41.3 $\pm$ 15.0 years (range, 4–90 years), males were slightly more common than females, and Asians (74.4%) were the predominant ethnic group. In another study of patients with disseminated TB, pre-existing immunosuppression (HIV, drug-induced) was significantly associated with ocular infection, while diabetes, age and gender were not [32]. 55.3% (26/47) patients in this cohort developed OTB. A recent study from South Africa compared ocular TB between HIV-positive and negative patients [33]. Consistent with studies in other EPTB, HIV-positive OTB patients more commonly had concurrent PTB (probable OTB), while HIV-negative patients did not (classified as possible OTB). High prevalence of OTB in patients with compromised immune status suggests that host immunity plays a crucial role in pathogenesis of the ocular infection.

Unlike the abundant data on role of bacterial factors in pathogenesis of PTB, there is lack of similar data for OTB. In a recent laboratory study, transcriptomic profiling of *Mtb* H37Rv infection of the human retinal pigment epithelium (RPE) cell line ARPE-19, revealed upregulation of *Mtb* genes involved in adherence, invasion, virulence and intracellular survival [34]. Two of the upregulated transcripts were also present in vitreous samples of patients with OTB. Further investigations into mycobacterial characteristics specific for ocular infection in clinical samples would be challenging, considering the rarity of microbiological evidence in human OTB.

3.2 Histopathological data on human ocular TB

The pathologic changes in OTB result primarily from hematogeneous dissemination and *Mtb* gaining access to uveal tract due to its rich blood supply. Despite the relative lack of tissue samples from eyes with OTB, there have been multiple reports on the histopathological

features of intraocular inflammation, associated with mycobacterial infection. The majority of these reports are from the pre-antibiotic era, when untreated ocular infection would lead to progressive inflammation and end-stage eye disease that required enucleation of the eyeball. Recently, histopathological data on a large series of 42 eyes collected over a period of 75 years (mostly in the pre-antibiotic era) was published [9]. In general, studies reveal that the organisms could lodge in different sites in the eye and ocular adnexa, though choroidal localization is most common. In addition to the choroid, severe inflammation can also involve the iris, ciliary body and retina, recognized clinically as panuveitis. Extensions of the choroidal inflammation into the retina manifests as chorioretinitis, retinal perivasculitis, neuroretinitis and vitritis. Lodging of the microbe in the anterior uvea results in iridocyclitis, pars-planitis and anterior uveoscleritis.

An overview of pathological changes in different anatomical sites of inflammation is given in Table 3. Choroiditis can present with clinical and pathologic changes in three morphologic features: unifocal conglomerate granuloma, multifocal granulomatous lesions or diffuse uveal granulomatous inflammation [9]. Conglomeration of granulomas forms a nodule with either multifocal necrosis or large area of necrosis surrounded by zones of epithelioid cells and lymphocytes (Figure 2). The multifocal tuberculous choroiditis is characterized by the presence of several round or oval elevated lesions distributed in the choroid and can also occur in the periphery of the choroid. Overlying choriocapillaris and RPE including the outer retina can be involved in the inflammatory process. These lesions reveal granulomas with central caseous necrosis and involve the inner and mid choroid with presence of a lymphocytic infiltration in the outer choroid [9,35-38]. The vitreous can display the presence of epithelioid histiocytes, lymphocytes and occasional giant cells. The retinal veins may show presence of lymphocytic infiltration in the outer wall, clinically recognized as perivasculitis.

Retinal involvement in the form of retinitis can be from extension of the choroidal inflammation into the retina. In such cases, it follows RPE and choriocapillaris damage. The damaged RPE cells can reveal the presence of intracellular acid-fast bacteria in the absence or detectable bacteria in the choroidal or retinal necrotizing inflammation [39]. Retinal granulomas without choroiditis are also reported but such lesions are rare. Here, the granulomas are seen adjacent to retinal vessels. In a recent histopathological report, retinal granulomas were found to be composed of epithelioid histiocytes without demonstrable presence of AFB [40]. The retinal vascular involvement, clinically labelled as retinal vasculitis or periphlebitis, represent primarily a mantle of lymphocytes in the outer walls of veins and venules. Such inflammatory infiltration primarily represents perivasculitis without occlusion of vessel lumen. The retina adjacent to veins can display focal tiny granulomas and rarely the granulomas involve vessel walls [41]. This report did not evaluate the choroid, as a retinal biopsy was taken from a patient and not from enucleated eye. However, earlier reports that included choroidal sections have also shown retinal granuloma with no cellular infiltration of the choroid [38,41].

Serpiginous-like choroiditis also known as multifocal serpiginoid choroiditis, consists of inner choroidal necrotizing granulomatous inflammation involving choriocapillaris, Bruch's membrane and overlying RPE and photoreceptors. The latter initially reveal disruption of inner and outer segments, followed by loss of photoreceptor cell bodies. The serpiginoid pattern is from irregular extension of the necrotizing inflammatory focus involving the inner choroid, choriocapillaris and RPE. In a recent report of a patient with serpiginous-like choroiditis with paradoxical reaction, histopathology of the retina and choroidal biopsy showed granulomatous inflammation with caseous necrosis involving inner choroid, Bruch's membrane and choriocapillaris [42]. Acid-fast bacteria could not be detected by Ziehl-Neelsen or Fite stains. The absence of staining for acid fast bacteria could be from robust immune response against the bacteria eliminating the infectious agent or the low sensitivity

in detection of the bacteria by the histologic strains or alternatively may be from a non-resolving autoinflammatory-type response after the eradication of the bacteria [43].

3.3 Mycobacterial dissemination to the eye and putative host responses

Since we have unequivocal evidence of *Mtb*-induced granulomatous inflammation in OTB, it can be safely presumed that *Mtb* disseminates to the eyes from the lungs, as in other forms of EPTB [20]. The dissemination of *Mtb* to the eye can be understood by drawing parallels from other forms of EPTB, especially CNS TB [44]. *Mtb* entering the lungs through inhalation of infected droplets is phagocytosed by alveolar macrophages [45]. These attract and colonize additional macrophages and dendritic cells (DCs) at the initial site of infection (Ghon focus). Infected DCs migrate through lymphatics to the regional lymph nodes where antigen presentation leads to generation of the adaptive immune response (in about two weeks). While this may or may not lead to control of primary infection in the lungs, infected DCs may enter the blood stream and can disseminate to various organs [21,46], including the eye. Infected DCs from the lungs need not be the only source of *Mtb* to the eyes. Progenitor DCs in the bone marrow carrying *Mtb* infection can also be released into the peripheral circulation [47], and get possibly seeded into the eye.

Mycobacterial dissemination to intraocular tissues has also been studied in various animal models. In one of the early studies, live or killed (by boiling for one hour) *M. bovis* were injected into the internal carotid arteries of **rabbits** [48]. The dead bacilli were injected as clumps and not as fine emulsion such that they could be trapped in the ocular circulation. It was noted that 70% animals developed ocular inflammation following injection of dead bacilli, compared to 100% in those injected with live bacilli. Nonetheless, both live and dead bacilli produced extensive intraocular inflammation, with similar lesions in the iris and choroid, including formation of caseating granuloma. More recently, **guinea pigs** exposed to aerosolized *Mtb*, the natural route of infection in the host, resulted in development of

granulomatous uveitis, caseating granulomata and demonstrable presence of acid-fast organisms in the eye [49]. In untreated animals, 42% (5 of 12) developed ocular infection with granulomatous inflammation. However, in animals treated with 3-drug anti-tubercular therapy (ATT), starting 2 weeks after the aerosolized *Mtb* exposure, none of the eyes showed acid-fast bacilli or granulomatous inflammation. In a **mouse** model of paucibacillary OTB, 12 *Mtb* genes were found to be significantly upregulated in the eye, and five of these were also identified in vitreous fluid of human OTB [50]. Finally, in a **zebrafish** embryo model of *M. marinum* infection, fluorescent tagged mycobacteria injected into the caudal veins of embryos could be tracked to the eye, where they formed early granuloma in the vicinity of inner and outer blood retinal barriers (Figure 3) [51]. Together, these animal experiments provide clear evidence of the ability of mycobacteria to disseminate hematogenously to the eyes.

The subsequent course of ocular infection can at best be speculative, based on existing knowledge on the distribution of immune cells in choroid and retina, and of CNS TB pathogenesis. Broadly, the infection can follow one of the two paths depending on its relationship with the blood retinal barriers (BRBs) [43]. Notably, the BRBs comprise of not only a physical barrier (endothelial tight junctions and RPE), but also an immunological barrier that includes the retinal endothelium, perivascular macrophages, pericytes, microglia, and the glia limitans [52]. Infected DCs outside the BRBs, such as those passing the fenestrated capillaries of the choroid may become lodged in the choroid and initiate focal inflammation. *Mtb*-infection that crosses the inner and outer BRBs is more difficult to explain. As in the case of CNS TB [44], the crossing over could happen transcellularly through the vascular endothelium or through infected DCs (Trojan Horse mechanism) [52]. In the perivascular space, the blood-borne infected DCs themselves or perivascular macrophages located between the endothelium and glia limitans could ‘licence’ T-cells to cross the BRB. In the context of neuroinflammation, activated T-cells have been shown to cross the glia limitans to reach the retinal parenchyma [53]. This requires further positive migratory signals

from the astrocytes forming the glia limitans, as well as surrounding cells [54]. Alternatively, *Mtb* antigens in the sub-retinal space could be sensed by 'periscope-like' processes of choroidal DCs, leading to formation of localised lymphocyte aggregates that manifest as retinochoroiditis. If the cell death is excessive, then this appears histopathologically as necrosis or caseation, although such immunopathology is relatively rare in the eye [10]. In addition to the above cell types, the RPE layer also forms an active interface between the retina and the immune system/ peripheral circulation by virtue of intercellular tight junction presence. These cells express MHC-class II antigens [55], as well as various Toll-like receptors required for phagocytosis of the bacteria and innate immune responses [56]. In an *in vitro* study, human RPE cells were found to have similar ability for phagocytosis of *Mtb* H37Ra as THP-1 macrophages, a human cell line [57]. In another study, RPE cells were found to control intracellular growth of *Mtb* (H37Rv) with similar efficacy to the macrophages [58].

3.4 Reactivation or progression of primary disease

The standard narrative for prevalence of TB is that one-third of the world's population is latently infected with *Mtb*, and 5-10% of them known to develop active disease from reactivation of the dormant organisms [59]. However, this dogma has been challenged in recent times, albeit in the context of PTB [60]. The authors reanalyzed historical evidence and concluded that a large proportion of TB cases result from progression of primary infection following recent transmission, rather than reactivation. They suggested that such recent transmission or reinfection would be particularly applicable to high TB-incidence countries, while reactivation could play a major role in low incidence countries. As in other areas of TB research, the timeline of disease progression has not been widely investigated in EPTB [61], much less in ocular TB. The challenge in EPTB is that the progression from the transmission event to disease does not happen directly but is interrupted by an intermediate stage of infection (with or without disease) in the lungs. As the exact

mechanisms of dissemination of *Mtb* from lungs to extrapulmonary organs remains unclear, it will be difficult to determine if the extrapulmonary disease occurred due to recent transmission of *Mtb* from the lungs or due to reactivation of organisms previously lodged in the extrapulmonary sites from prior pulmonary infection. An early study from the pre-antibiotic era reported that the primary complex in lungs rarely progresses after one year of infection and gets completely calcified by three years [61]. Interestingly, a comparison between time to onset of PTB and EPTB following TB contact showed that the EPTB had a much lesser likelihood of onset within the first five years after contact (23.7%) as compared to PTB (72.6%) [62]. Another study showed that patients with history of TB contact in past two years were less likely to have EPTB than PTB [63]. A third study found that the likelihood of recent contact with PTB varied according to the site of EPTB, being significantly higher with pleural than lymphatic TB [64]. Since the onset of EPTB is temporally separated from TB contact in most studies, the possibility of reactivation in the extrapulmonary organ appears to be more likely than that of progression from primary disease in cases of EPTB. This is likely aided by the fact that *Mtb* can persist as latent infection in various anatomical and cellular niches of the body [47].

Regardless of the exact mechanism of onset of EPTB/ OTB (recent transmission or reactivation), it is imperative to recognize that the host immune response can potentially clear *Mtb* infection from the body [65]. There is evidence from clinical studies that immunoreactivity to *Mtb* antigens can persist even after clearance of infection [66,67]. In fact, longer the duration of infection prior to clearance, more robust the immunological memory and greater the chances of it persisting after the clearance [68]. This has implications on interpretation of diagnostic tests involving immunoreactivity to mycobacterial antigens (TST or IGRA), which are commonly used in the diagnosis of OTB. Both these tests are measures of immunological memory that develops following adaptive immune response to *Mtb*. Since immunological memory can persist even after clearance of infection, mere presence of a positive TST or IGRA, in the absence of other supporting clinical

evidence of disease should not be used for diagnosis of OTB. Interestingly, some studies have suggested that higher IGRA values are associated with lower risk of treatment failure with ATT in OTB [69,70]. It is possible that such high IGRA values in OTB patients are linked to the prolonged systemic infection prior to onset of ocular disease, as noted in other EPTB studies [62-64].

4. Clinical observations unexplained by infectious mechanisms

Despite the extensive literature supporting the infectious mechanisms of mycobacterial infection in the eye, several clinical observations on OTB remain unexplained. Foremost is the mismatch between the extent of intraocular inflammation and low detection rates of *Mtb* in ocular tissues and intraocular fluids [9,15]. As described above, intraocular inflammation in OTB is extensive and affects nearly all types of ocular tissues. It has been shown to cause moderate to severe visual impairment in up to 40% of affected eyes [4]. However, the detection rates of *Mtb* in intraocular fluids, on culture, microscopy or molecular diagnosis remain consistently low, despite the technological advancements. One possibility is that the organisms are located in deeper tissues such as retina and choroid that are not amenable to diagnostic evaluation. However, even in enucleated eyes, with extensive granulomatous inflammation and caseous necrosis, only 1-2 organisms were found in association with giant cells or areas of necrosis [9].

Second, despite multiple studies supporting a beneficial role of ATT in OTB [10,13,71], the response of OTB to ATT remains unpredictable. Nearly all forms of intraocular inflammation in OTB need adjunctive corticosteroid therapy either locally or systemically [3]. Specifically, in serpiginous-like choroiditis, inadequate steroids was associated with paradoxical worsening in nearly 15% of patients following initiation of ATT [72]. Even across all forms of OTB, a large retrospective study that grouped patients into treatment with ATT and corticosteroids or with corticosteroids alone, found that more than half (53.5%) of the

patients treated with corticosteroid therapy alone had no recurrence over a median follow up of 31 months (minimum 6 months) [13]. In the same study, nearly 16% of patients receiving ATT experienced recurrent or persistent intraocular inflammation even after 18-24 months of ATT. Together, these data suggest that additional pathogenic mechanisms, besides the primary infection, may play a role in development of OTB.

5. Indirect immune mechanisms in pathogenesis of ocular TB

Indirect immune mechanisms refer to those processes whereby *Mtb* plays a role but is unrelated to replication of live bacilli within the eye. The role of such immune mechanisms in inducing inflammation in OTB has long been suspected. As early as the 1940s, it was stated, "TB is not only the cause of ocular disease, in which tubercles can be demonstrated, but may be the etiological factor in a host of ocular inflammatory diseases of doubtful character, especially those of a recurrent or chronic type, even though a tuberculous origin cannot be demonstrated elsewhere" [73]. Woods first provided objective data on the likelihood of indirect immune mechanisms in OTB through experimental studies in rabbits [18]. He advocated independence of immunity and allergy in the host immune response to *Mtb*, and tried to explain the pleomorphism seen in OTB with Rich's law, given below:

$$\text{Lesion is directly proportional to } \frac{\text{No. and virulence of bacilli x allergy}}{\text{Resistance}}$$

According to this law, a large dose of bacilli with high tissue allergy, but low immunity (resistance) would result in a large lesion, while small number of bacilli with high resistance would result in a small lesion. However, in the case of phlyctenular conjunctivitis, long suspected to be tubercular, attempts at producing phlycten in BCG challenged rabbits with subconjunctival injections of tuberculin (purified protein derivative) did not yield any results [19]. Thus, the respective contributions of live/ replicating *Mtb* within the eye, and non-viable bacteria (or bacterial antigens), remains poorly understood in OTB. Furthermore, the difficulty of culturing mycobacteria and the limitations of molecular diagnostic tests from

ocular fluid samples [74], present a challenge in clearly delineating infection and immune-mediated mechanisms at a given time point, in the course of disease. Despite these limitations, data from experimental animal models and more recently, from human OTB can provide useful insights into the existence of the indirect mechanisms. Broadly, these could be classified into those involving the presence of dead/ non-viable *Mtb* or bacterial products in the eye; and those involving activation of autoimmune response in the host.

5.1. Experimental data supporting immune-mediated mechanisms

Experimental data supporting indirect immune mechanisms can be obtained from animal models of OTB as well as those of experimental autoimmune uveitis (EAU). In the **rabbit** model described above, intra-carotid injections of clumps of dead *Mtb* induced granulomatous inflammation in the iris, ciliary body and choroid, just as with live *Mtb* [48]. However, there was one major difference. No evidence of retinal inflammation such as retinal vasculitis was noted on injection of dead *Mtb*, most likely since dead bacilli failed to cross the blood-retinal barrier. Further evidence supporting the role of bacterial products in initiating intra-ocular inflammation can be obtained from ***in vitro*** experiments, where *Mtb* protein, Early Secreted Antigenic Target -6 (ESAT-6), and mycobacterial RNA (specifically double-stranded RNA) induced NLRP3 inflammasome dependent Caspase-1 activation in RPE cells [75]. The results were replicated when ESAT-6 was injected into the sub-retinal space in mice. These experiments demonstrated that mycobacterial components such as secreted proteins and nucleic acids, if transported to the eye, could potentially generate innate immune responses mediated inflammation, even in the absence of complete bacilli.

Animal models of EAU also support an indirect role of *Mtb* in initiating intraocular inflammation. **EAU** is a T-cell mediated autoimmune disease of the neural retina and surrounding tissues, induced by immunization with retinal antigens [76,77]. The retinal antigen is typically combined with Complete Freunds Adjuvant (CFA), which contains heat-killed *Mtb*, which provide the innate signals essential to polarize retinal-antigen specific T-

cells towards proinflammatory phenotype [78]. This role of heat-killed *Mtb* in generation of EAU can be extrapolated to possible remote immune priming of autoreactive T-cells in human uveitis, by mycobacterial antigens (from live or dead *Mtb*) located either in the eye or other organs. Further evidence of role of *Mtb* antigens (in the absence of viable bacilli) can be obtained from the model of primed mycobacterial uveitis in rats (also called experimental mycobacterial uveitis in rabbits) [79,80]. In this model, systemic priming of the animal by subcutaneous injection of *Mtb* H37Ra antigen, followed 7 days later by an intravitreal injection of the same antigen, induces an acute inflammation in the vitreous and anterior chamber (with sparing of retina and choroid). This model may recapitulate at least some forms of anterior (granulomatous and non-granulomatous) and intermediate uveitis, seen in patients with immunological (and/or radiological) evidence of past *Mtb* infection. Together, these data provide convincing evidence that non-viable *Mtb* or its products can initiate intraocular inflammation either through local activation of innate immune response or through priming of autoreactive T-cells.

5.2 Human data supporting indirect mechanisms: role of retinal autoimmunity in ocular TB

Recently, we reported the intraocular immune response from vitreous humor of patients with clinical diagnosis of OTB [81]. Our hypothesis was that intraocular T-cells in OTB would produce a cytokine response to mycobacterial antigens and not to retinal self-antigens. This would support the direct role of *Mtb* in pathogenesis of ocular TB. Remarkably however, the intraocular T-cells that were primarily $CD4^+$ T-cells, of effector and central memory phenotypes, were reactive to both *Mtb*-specific antigen, ESAT-6, and retinal crude extract (RCE) antigens (Figure 4). The RCE-reactive (autoreactive) cells showed greater abundance and cytokine production compared to ESAT-6 reactive cells. We did not find any sequence homology between ESAT-6 and four select retinal antigens. Not only that, the RCE-reactive cells were also resistant to activation-induced cell death (AICD), and therefore

likely to persist longer in the vitreous. Together, these results provided the first evidence of an autoimmune response contributing to inflammation in OTB, in conjunction with the anti-mycobacterial response. However, they also raised at least two important questions, that would need further clarification:

1. Are there indeed two different populations of T-cells in the eye, or is it that the same population is reacting differently to two different antigens (cross-reactivity)?
2. If there are two different populations, then do the autoreactive cells specifically contribute to the pathogenesis of OTB, or they are merely an epiphenomenon?

To rule out cross-reactivity, the ideal method would have been to sort each population of T-cells using tetramers labelled with either ESAT-6 or retinal antigens, and then demonstrate their lack of responsiveness to other antigens. However, the T-cell counts from vitreous fluid samples were not adequate for cell sorting experiments. As a result, only indirect evidence such as differential cytokine production and sensitivities to AICD were available to support the existence of two distinct populations. Unless proven by tetramer-based sorting, we can only speculate that both cross-reactivity and independent autoreactive processes could be responsible for the reactivity to both *Mtb* and retinal antigens. The second question, concerning pathogenicity of autoreactive T-cells, requires correlation between presence of autoreactive T-cells and disease severity or the long-term prognosis of the condition. However, the numbers of patient samples were not sufficient to draw these comparisons. Also, only 8 of 13 patients with OTB showed reactivity to RCE in vitreous samples. Thus, it remains unclear if the autoreactive T-cells merely have bystander presence in TB-associated inflammation (epiphenomenon), or they actually influence the course of disease.

While we proposed that disruption of the BRB led to ingress of autoreactive T-cells into the eye, it is possible that BRB disruption allows release of retinal antigens into the peripheral circulation and generation of the autoimmune response. However, we did not find any evidence of RCE-reactive cells in the peripheral circulation of patients with autoreactive cells in the vitreous. In line with this observation, antiretinal antibodies too have been found to be

lower in the peripheral circulation of uveitis patients with evidence of systemic TB infection, as compared to non-TB uveitis patients [82]. Surprisingly however, systemic autoreactivity (anti-nuclear antibodies) is high in active systemic TB [83] and organ-specific autoreactivity (anti-retinal antibodies) is high in non-TB uveitis [84]. In contrast, regulatory T-cells (Tregs) in the peripheral circulation have been found to be decreased and functionally hyporesponsive in patients with OTB [85,86]. Similar decrease in frequency and function of Tregs has also been reported in another uveitis entity, Vogt-Koyanagi-Harada syndrome [87]. Taken together, the possibility of these autoreactive cells having only a bystander role in the vitreous appears less likely. Future studies recruiting patients with different grades of ocular inflammation and duration of disease, will help in determining the pathological role of autoreactive T-cells in OTB.

6. Lessons from autoimmunity in systemic TB

The concept that autoimmune phenomena may be augmenting inflammation in OTB is consistent with a recently advanced hypothesis that the induction of an autoimmune or auto-inflammatory process plays a central role in the immunopathology of pulmonary TB [88]. Indeed, a key precipitant of this hypothesis was the necessity to treat patients of OTB with both ATT and corticosteroids even in the absence of direct evidence of ocular infection, and the association of uveitis with TB and other typical autoimmune disorders such as Behcet's disease, ankylosing spondylitis and sarcoidosis. Since that publication, further evidence has emerged both from basic science and clinical experience supporting autoimmunity in systemic TB (Table 3). Furthermore, other investigators have proposed that "loss of tolerance" to *Mtb* is a key component in the development of active TB [89-90], which follows similar conceptual lines, arising from their observations of the critical role of regulatory T cell responses in the mouse model of TB [91].

6.1 Adverse effects of excessive inflammation in TB

Both Koch and Virchow recognized that the host immune response to *Mtb* was a double-edged sword, being responsible for control of the pathogen but also leading to tissue destruction and transmission [92]. Further support for the harmful effect of an excessive immune response came from Comstock's seminal epidemiological studies, where analysis of tuberculin reaction from 82,000 children showed that a greater immune response to mycobacterial antigens in childhood associates with a greater chance of developing pulmonary disease after puberty [93]. However, the precise nature of this harmful immune response has remained elusive [94], hindering the development of novel therapies.

The proposal that an autoimmune process may be a key component emerged from clinical and experimental observations that do not conform to current disease paradigms, whereby an inadequate immune response is thought to be responsible for the development of active TB (Table 4). For example, patients with TB frequently have circulating autoantibodies typical of autoimmune disease and develop phenomena such as erythema nodosum and uveitis that are common to TB and autoimmune diseases [95-96]. Furthermore, TB and sarcoidosis are often histologically indistinguishable [88]. Sarcoidosis is a corticosteroid-responsive disease of unknown etiology, but given the histological similarities to TB, a common underlying process seems highly likely to be driving granuloma maintenance in both diseases. Experimentally, TB-derived antigens in Freund's adjuvant are used to cause autoimmune disease in mice, whereas immunosuppression with azathioprine prevents cavitation in rabbits [78].

Experimental investigation into a potential autoimmune process in pulmonary TB is challenging, as the antigen is unknown and indeed autoimmunity may not be driven by a single antigen. Further support for an overlap of fundamental processes in TB and autoimmune disease came from analysis of gene expression signatures in circulating immune cells (Figure 5) [97]. Comparison of infectious disease, autoimmune disease and TB showed that there was a greater overlap between autoimmune disease and TB than

between TB and other infections. In addition, once all common genes were accounted for, only a small minority were exclusive to TB. This again suggests that TB results from processes driven by both infection and autoimmune processes.

6.2 TB reactivation and immune checkpoint inhibition

Surprisingly, support for an autoimmune process causing pathology in TB has emerged from the cancer field. Immune checkpoint inhibition is rapidly emerging as a transformative approach to diverse malignancies, by permitting a more effective immune response to malignant cells and therefore improving outcome [98]. The importance of the discovery of these pathways was recognized by the award of the Nobel prize in 2018. The main side effects of either programmed death - 1 (PD-1) or Cytotoxic T Lymphocyte-associated Antigen (CTLA-4) inhibition are primary immune-related adverse events (irAEs), such as skin rash, colitis, pneumonitis or hypophysitis, which require treatment with systemic corticosteroids to reduce inflammation [99]. These events could be regarded as autoimmune inflammation or alternatively loss of tolerance to antigens that previously did not precipitate inflammation. An alternative explanation is that checkpoint inhibition modulates the balance between T regulatory and pro-inflammatory T cells to lead to immunopathology [100].

In terms of control of *Mtb*, the generic activation of the immune response by these agents would be predicted to control infection, and indeed immune checkpoint inhibition has been proposed as a host-directed therapy for TB [101]. However, and counter-intuitively, a rapidly accumulating number of cases of TB associated with immune checkpoint inhibition are being reported in the literature [102-103]. Consistent with this observation, CTLA-4 genotype associates with TB severity [104]. Taking the phenomena of immune checkpoint inhibition causing autoimmune-like adverse events and an increase in TB reactivation together supports the concept of an autoimmune process contributing to pathology in human TB.

6.3 Underlying mechanisms of autoimmune inflammation

A central outstanding question is whether the development of an autoimmune process in TB is merely an epiphenomenon related to a chronically activated immune system, or a specific evolutionary strategy of *Mtb*. If the latter, it seems likely that novel treatment interventions will need to consider the effect of autoimmune inflammation, in particular for the recently advanced approach of host-directed therapy [105], and to inform novel vaccination approaches. Humans and *Mtb* are thought to have co-evolved since the migration out of Africa approximately 70,000 years ago [106], and therefore it seems plausible that the pathogen has evolved to cause autoimmune phenomena as an evolutionary strategy.

TB must cause extensive pulmonary pathology to drive cavitation that leads to transmission (Figure 6), overcoming the anti-inflammatory environment of the lung that protects from excessive cellular infiltration and preserves the core function of gas exchange [107]. As bacillary load is very low in the granulomas [108], induction of additional inflammation by uninfected cells expressing host stress antigens represents an alternative strategy for *Mtb* to cause the lung destruction necessary for transmission. This would avoid exposing the *Mtb*-infected macrophages to a potentially efficacious host immune response. It can be hypothesized that the antigens are likely to be lipids, as several group 1 CD1-presented lipids are common to both the pathogen and host [109] and are associated with other autoimmune diseases [110]. Currently, we have insufficient evidence to definitively prove that there is autoimmune inflammation contributing pathology in human TB, only circumstantial evidence (Table 3). Therefore, with the current state of knowledge it is impossible to determine the relative contribution of inflammation driven by the pathogen compared to that which is autoimmune in nature, nor whether it is necessary to have pathogen-derived molecules at each site to initiate a response, or if generic shared stress ligands suffice. This is particularly pertinent in the context of ocular TB.

6.4 Linking systemic autoimmunity in TB to the eye

If ocular inflammation is an off-target manifestation of an evolutionary strategy of *Mtb* to cause lung damage, the treatment implications primarily relate to effective strategies to limit further inflammatory damage to improve outcome. Ultimately, this will only come with understanding the basic pathological mechanisms. We propose that studying the host immune response both at the site of disease (eye and lung) and systemically by studying circulating peripheral blood mononuclear cells will be necessary to gain the mechanistic understanding required to inform more targeted therapies to reduce excessive inflammation in TB-related ocular inflammation.

Tangential support for a remote priming event comes from the recently described phenomenon is uveitis associated with immune checkpoint inhibition treatment for disseminated malignancy [111]. This suggests that systemic immune dysregulation is sufficient to cause uveitis in these patients, in the absence of exogenous pathogen-derived antigen. These adverse events suggest that retinal antigens within the eye that are usually tolerated become inflammatory in the absence of inherent physiological restraints on the immune system. Consequently, in the context of OTB, the inflammatory response may be due to either local disease or alternatively distal inflammatory priming then affecting the eye. Detailed immunological investigation will be required to define the relative contribution of each process.

7. A unified model for ocular TB pathogenesis and future directions

OTB represents a wide variety of clinical phenotypes that may not be explained by a single unified model of pathogenesis. However, classification of clinical phenotypes of OTB into two groups, depending on whether the BRB is breached or not, may help in assigning common pathways for each group. This model of classification was recently proposed for pathogenesis of uveitis in general [43]. The authors noted that any compromise in BRB is always associated with uveal inflammation, but not all uveal inflammation need compromise

the BRB. The significance of breach in BRB is that it will determine if the ocular inflammation is also associated with autoimmune response to retinal antigens. Considering that TB has been associated with nearly all forms of uveitis and that there is evidence of local autoimmunity in OTB, we propose that the above classification could be applied to OTB as well. Apart from breach in BRB, the other expected determinants of clinical phenotype would be localization of infection in the eye, dose of inoculum and virulence/viability of organism.

7.1 Ocular TB *without* breach in BRB

This would include anterior uveitis, intermediate uveitis and deeply located choroiditis (unifocal or multifocal) that has not involved the RPE. In these phenotypes, we expect that dissemination of *Mtb* from pulmonary or extrapulmonary sites of infection to the eye would lead to an *Mtb*-specific adaptive immune response in the eye, resulting in the formation of a granuloma. Alternatively, as demonstrated in animal experiments with heat killed *Mtb*, even mycobacterial products (secreted proteins or nucleic acids) that get translocated to the eye can generate intraocular inflammation presumably through innate immune activation of myeloid cells (autoinflammation). The severity of ocular inflammation in these cases, as shown in animal studies, will probably be determined by viability of bacilli, inoculum in the eye, and virulence of organisms. Thus, killed *Mtb* or live *Mtb* in small numbers would probably induce low grade inflammation, as will low virulence organisms such as *M. bovis BCG*.

7.2 Ocular TB *with* breach in BRB

Breach in BRB would be expected in two of the common presentations of OTB: retinal vasculitis and serpiginous-like choroiditis. The former would cause disruption of the inner BRB (vascular endothelium) while the latter, since it affects the superficial choroid and RPE, would affect the outer BRB. In either case, disruption of BRB would result in autoimmune response either by allowing access of autoreactive T-cells in the circulation to their cognate antigens in the retina, or by release of retinal antigens into the peripheral circulation. As

noted in the section on role of autoimmunity, these autoreactive T-cells are resistant to AICD and can potentially prolong the inflammatory response and/or cause recurrent inflammation. Alternatively, *Mtb* or its products can function as local adjuvants, either in the eye, or at an extraocular site to facilitate activation of retinal antigen-specific autoreactive T-cells and thereby autoimmune uveitis. An open question is what the precise sequence of events is, and whether ongoing *Mtb* antigens are required to maintain inflammation, or whether once an initial inflammatory focus develops it can self-sustain in a similar way that sarcoid granulomas can persist. An outline of possible pathomechanisms in OTB with breach in BRB is depicted in Figure 7.

8. Future directions and conclusions

The most important challenge to further advance the field would be to determine how each of these mechanisms combine together during the development of individual phenotypes of OTB. This would have significant implications on both diagnosis and treatment of this condition. One approach could be to determine if the relative proportions of *Mtb*-specific and autoreactive T-cells vary in different clinical phenotypes of OTB. While our earlier approach involved investigating cytokine response to *Mtb* or retinal autoantigens, future studies could be more specific by application of HLA-matched MHC class II tetramers [112], or CD1 lipid-loaded tetramers [113], for identification of *Mtb*-specific CD4+ T-cells. Alternatively, high-throughput sequencing of T-cell receptor-β (TCR-β) genes can be done in paired samples of vitreous and blood to demonstrate differences between the two compartments and enrichment of mycobacterial and/or autoreactive sequences inside the eye. TCR repertoire in different clinical phenotypes of OTB can provide insights into the ontogeny of the disease [114,115]. Together, these strategies should be able to inform more accurate diagnostic and therapeutic approaches to different subsets of OTB. Thus, in bacteria-driven phenotypes the diagnosis and treatment would focus on recognition and elimination of *Mtb* from the eye,

while in autoimmunity-driven phenotypes, the focus would be on control of the inflammatory response.

The information gained from immunological studies will also be able to delineate the true spectrum of OTB. This might lead to inclusion of previously unrecognised entities, and/or exclusion of some of the currently accepted phenotypes from OTB. In conclusion, OTB has varied pathomechanisms that likely work in tandem for the development of different clinical manifestations of the disease. Comprehensive understanding of the process will facilitate accurate diagnosis and treatment of this condition.

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Figure legends

Figure 1: Clinical phenotypes of ocular TB. (A) Serpiginous-like choroiditis or multifocal serpiginoid choroiditis of the right, with healed pigmented scarring in the center and yellowish-white creamy lesions along the temporal margins. Active skip lesions are also

seen beyond the margins of the main lesion. (B) Retinal periphlebitis along the supero-temporal arcade of right eye, associated with focal retinitis lesion overlying the blood vessel (arrow). (C) Solitary tuberculoma of the right eye involving the temporal macula (D) Optic neuritis of the left eye with optic disc edema, peripapillary hemorrhages, vascular tortuosity and surrounding retinal edema.

Figure 2: A 25 year-old male presented with complaints of sudden loss of vision in right eye for 2 months. He also gave a history of low-grade fever 8 months ago which persisted for a month. He was investigated during the time of febrile illness and a diagnosis of tuberculosis was made based on the positive skin test (with an induration of 20 mm). An empirical anti-TB treatment (ATT) was started. The patient developed sudden decrease of vision in right eye after one month of taking the ATT, after which he was started on a multi-drug resistance (MDR) ATT regimen. (A) Slit-lamp examination of right eye showed circumciliary congestion, shallow anterior chamber, aqueous cells 2+, flare2+, and festooned pupil with neovascularisation of the iris and total cataract with vascularisation. (B) Anterior chamber tap of the right eye was polymerase chain reaction (PCR) positive for MPB64 DNA sequence of *Mycobacterium tuberculosis* (*Mtb*). MDR ATT was continued, but the condition worsened after 1 month, when the eye was enucleated for gross and histopathological evaluation. (C) Gross photograph of the horizontal cut section of globe showing sub-retinal whitish mass. (D) Microphotograph of the paraffin section of the enucleated globe showing caseation necrosis (star) with degenerated polymorphs and multiple cholesterol clefts were seen in the vitreous cavity. Ziehl-Neelson stain showed no acid-fast bacilli, but PCR of paraffin-fixed section were positive for Mtb sequences MPB64 and IS6110 on nested PCR. (Figure courtesy of Dr. Jyotirmay Biswas, Sankara Nethralaya, Chennai, India).

Figure 3: Granuloma formation (arrow) in transgenic (*mfap4:tdTomato*) zebrafish with red fluorescent macrophages and infected with green-fluorescent *Mycobacterium marinum* (*Mm*). The *Mm* were injected at 4 days post fertilization at a dose of 25 colony forming units

per μL into the caudal vein of embryos. Scale bars, 100μm in 1-dpi image and 50μm in others.

Figure 4: Early Secreted Antigenic Target-6 (ESAT-6) and retinal crude antigen (RCE)-specific $CD4^+$ T cells coexist in vitreous humor of ocular TB patients. (A) $CD4^+$ T cells from vitreous humor of ocular TB patients were stimulated with ESAT-6, RCE, and skin crude extract (SCE) (10 lg/mL each) along with anti-CD28 (2 lg/mL) for approximately 12 hours along with 10 lg/mL Brefeldin A and/or 2 lmol/mL monensin during the last 8 hours. Cells were fixed and stained for TNF-α, IL-17A, IFN-γ, and IL-10 (n = 6). All flow cytometry figures represent single-patient data. (B) Comparison of cytokine responses, after activation with ESAT-6 and RCE. Cytokine percent positive cells were compared by using paired t-test. Data are shown as mean percentages. Mean ± SD (n = 6). P≤ 0.5 was considered significant. *P≤ 0.05. Reprinted with permission from Investigative Ophthalmology and Vision Science. Copyright 2017 The Authors. Tagirasa et al., 2017. Autoreactive T Cells in immunopathogenesis of TB-associated uveitis. Invest Ophthalmol Vis Sci. 58:5682–5691.

Figure 5: Venn diagram showing analysis of differential gene expression in circulating immune cells in tuberculosis compared autoimmune and infectious diseases. A large number of genes are common to all conditions, reflecting a generic inflammatory response. However, the remaining genes modulated in tuberculosis have greater communality with autoimmune disease than infection, implying common pathological mechanisms. Values further filtered by a fold change of >2 are shown exclusive to tuberculosis. Reprinted with permission of the American Thoracic Society. Clayton et al., 2017. Gene expression signatures in tuberculosis have greater overlap with autoimmune diseases than with infectious diseases Am J Respir Crit Care Med 196 655-

656. The American Journal of Respiratory and Critical Care Medicine is an official journal of the American Thoracic Society.

Figure 6: Thoracic computerized tomography of a patient with pulmonary tuberculosis. Extensive lung inflammation develops, within which air-filled cavities develop. Patients with cavitary disease are the most highly infectious and drive transmission.

Figure 7: Flowchart showing *possible* pathomechanisms of ocular TB associated with breach in blood-retinal barrier. Adapted with permission from Investigative Ophthalmology and Vision Science. Copyright 2017 The Authors. Tagirasa et al., 2017. Autoreactive T Cells in immunopathogenesis of TB-associated uveitis. Invest Ophthalmol Vis Sci. 58:5682–5691.

Figure 1

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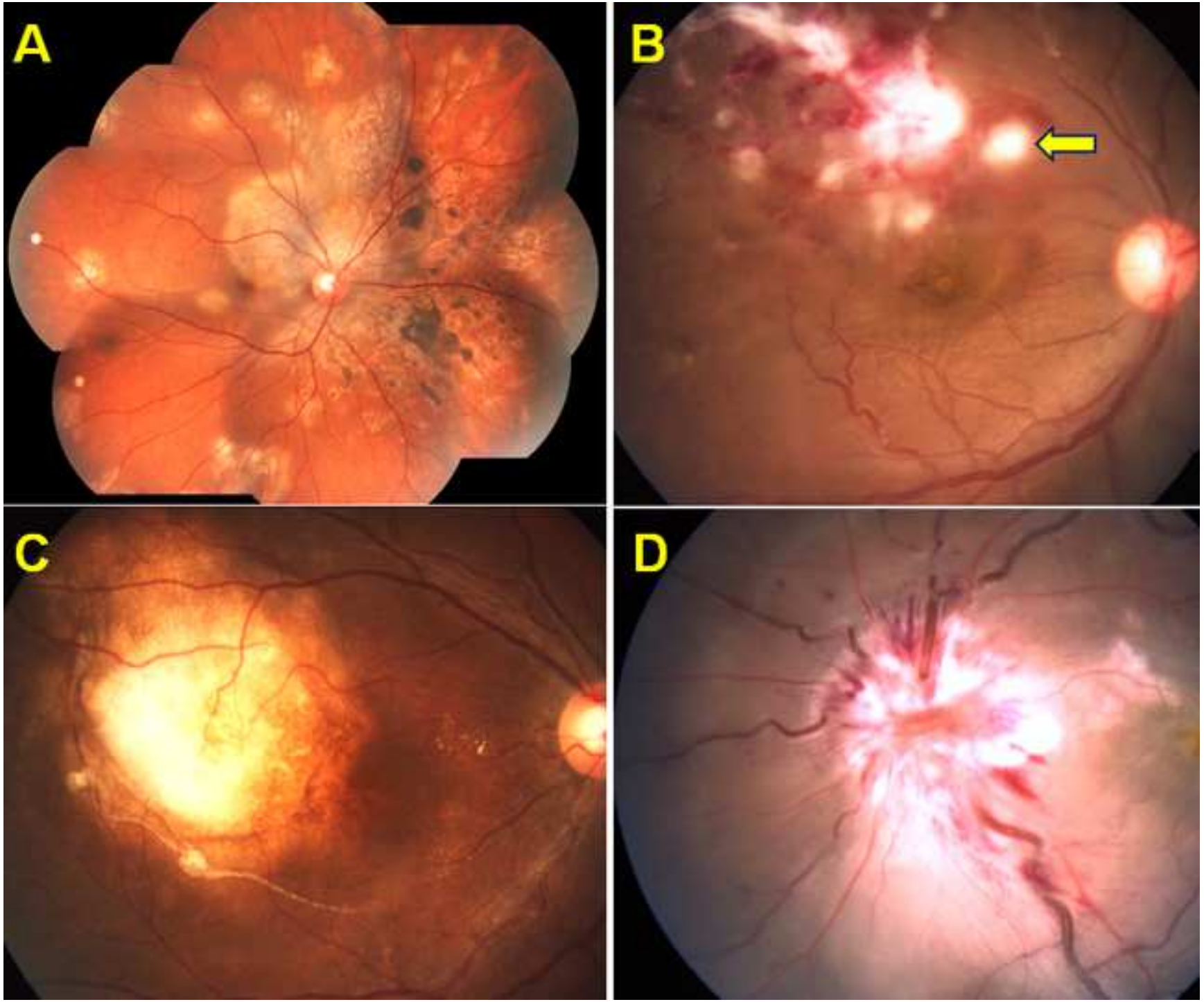

Figure 2

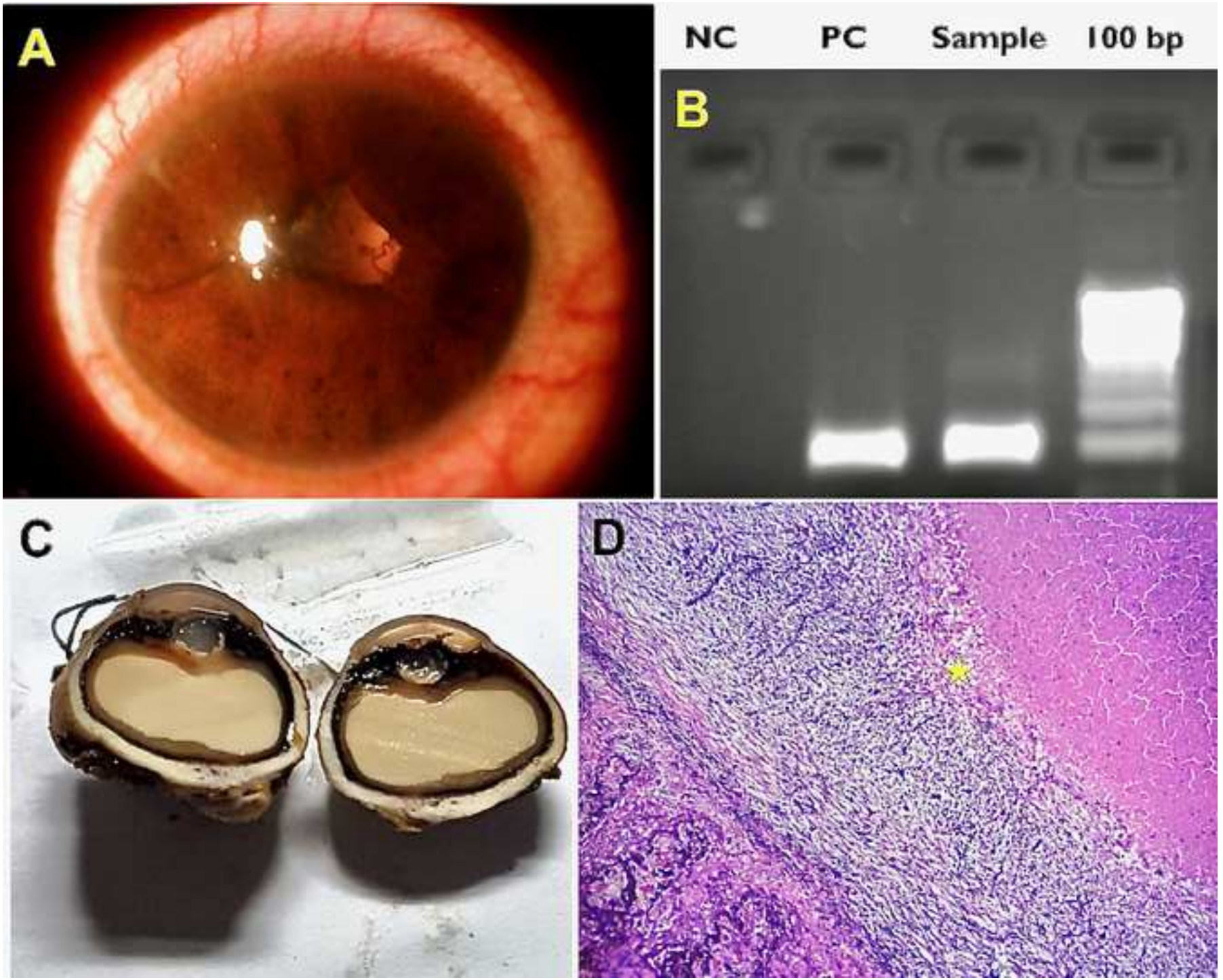

Figure 3

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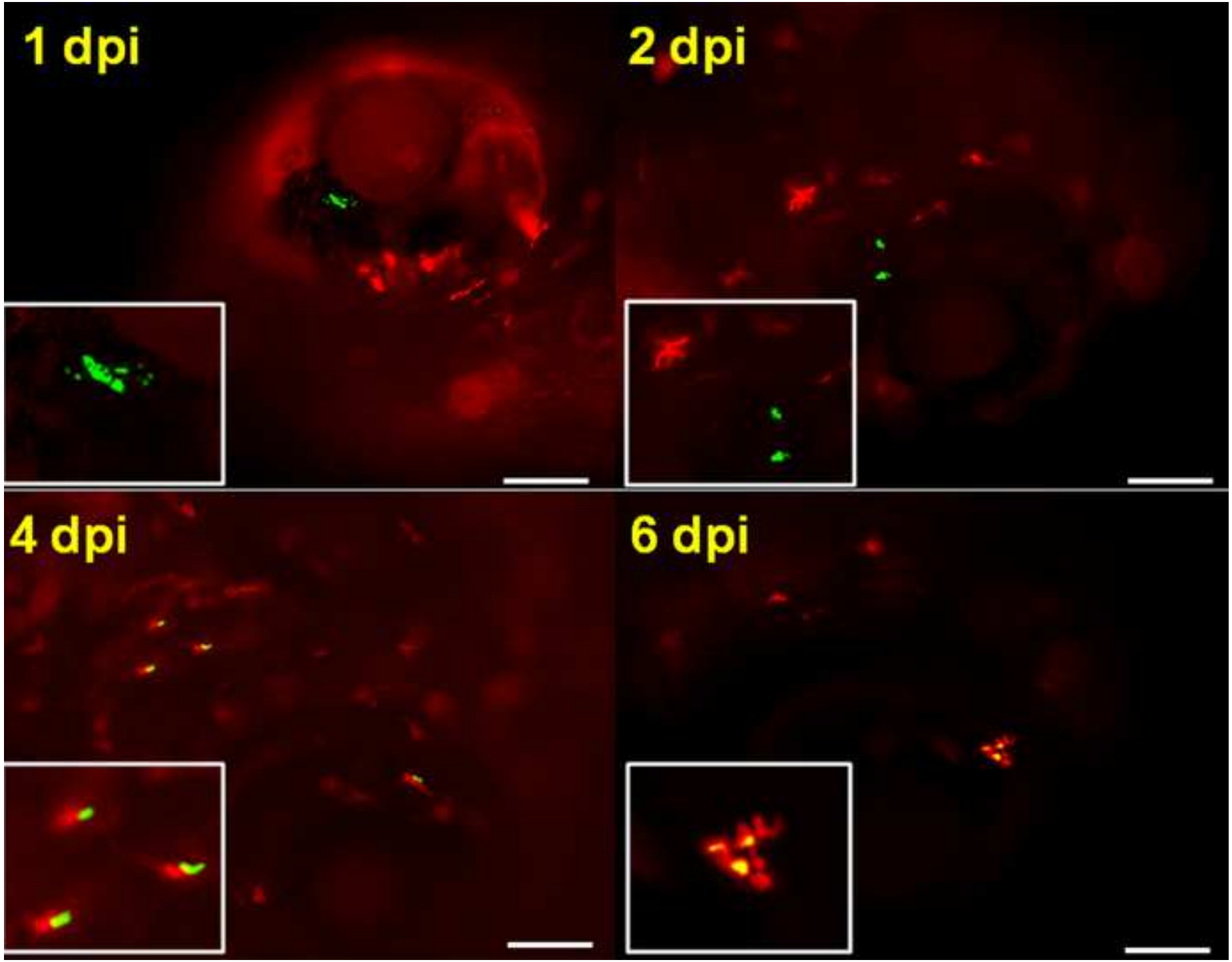

Figure 4

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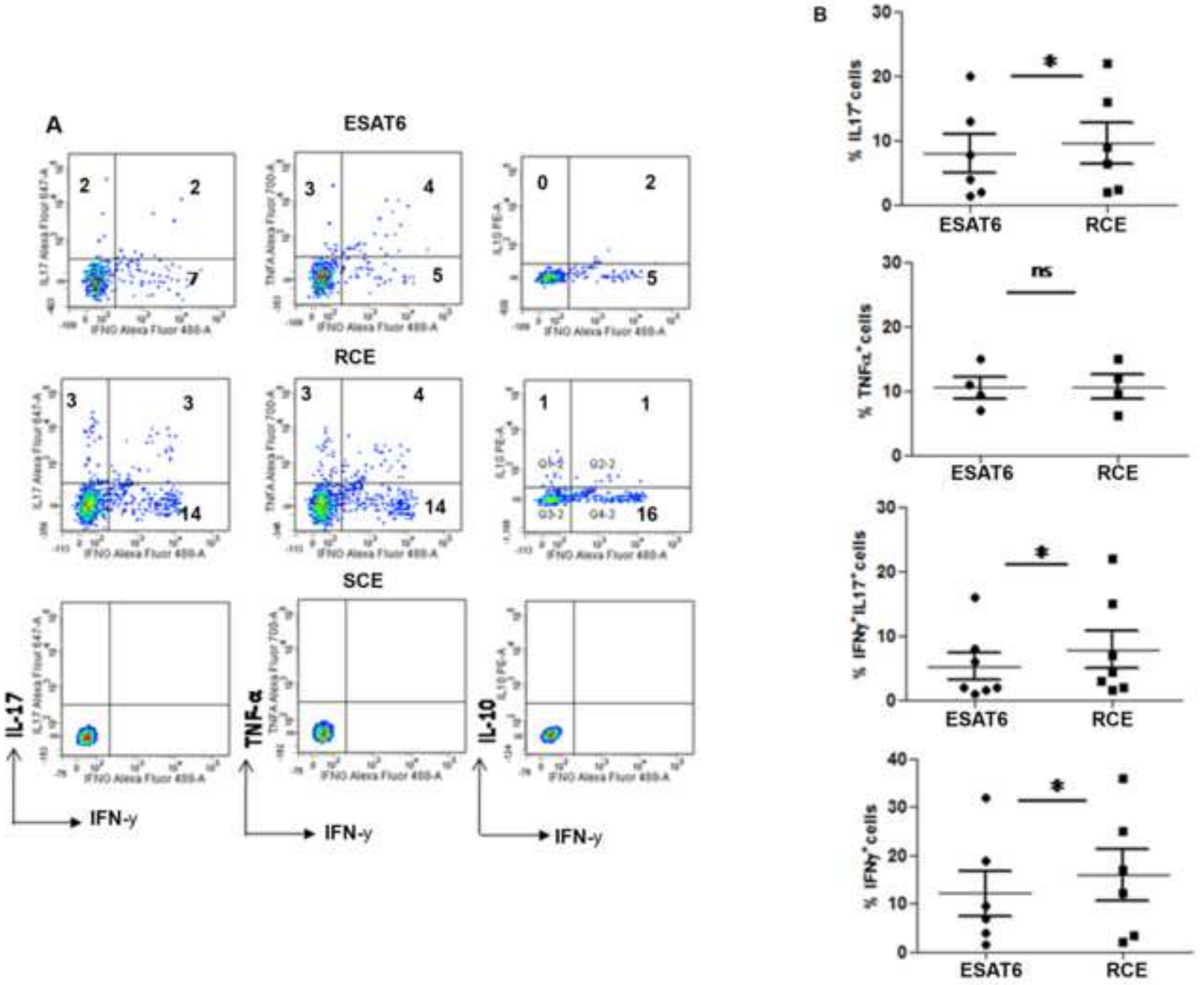

Figure 5

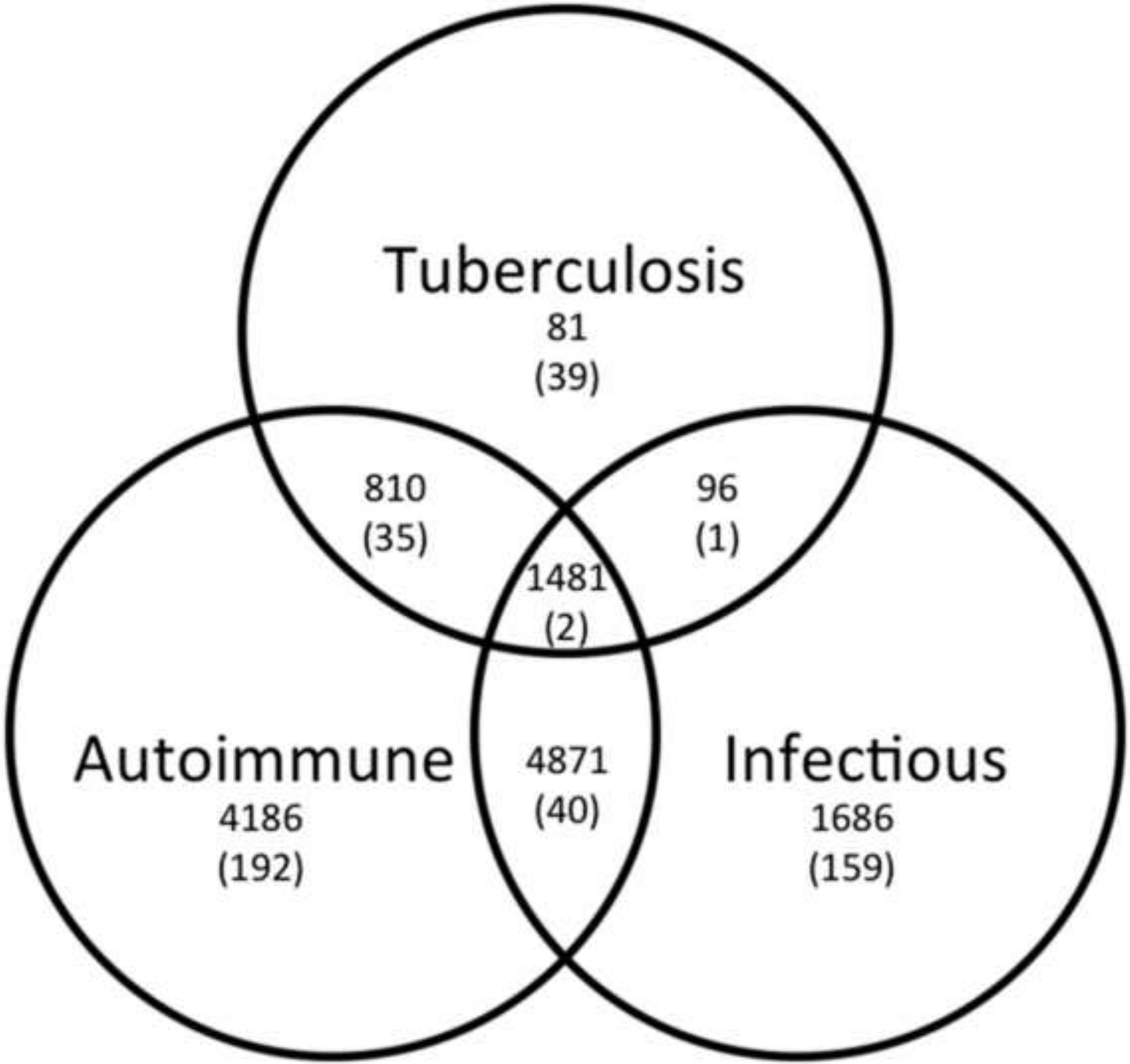

Figure 6

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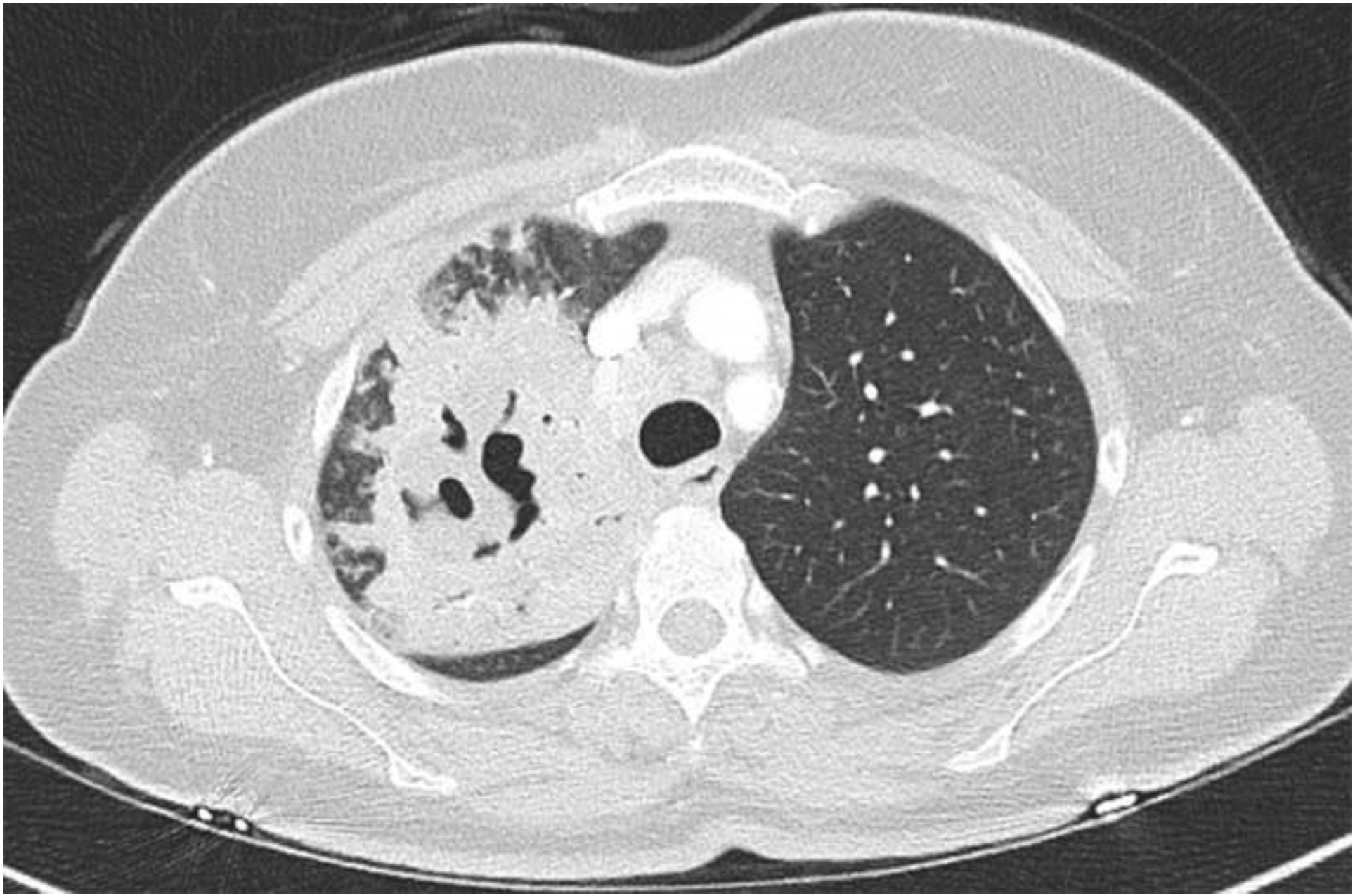

Figure 7