REVIEW

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A Tolerogenic Role of Cathepsin G in a Primate Model of Multiple Sclerosis: Abrogation by Epstein–Barr Virus Infection

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Abstract
Using a non-human primate model of the autoimmune neuroinflammatory disease multiple sclerosis (MS), we have unraveled the role of B cells in the making and breaking of immune tolerance against central nervous system myelin. It is discussed here that B cells prevent the activation of strongly pathogenic T cells present in the naïve repertoire, which are directed against the immunodominant myelin antigen MOG (myelin oligodendrocyte glycoprotein). Prevention occurs via destructive processing of a critical epitope (MOG34-56) through the lysosomal serine protease cathepsin G. This effective tolerance mechanism is abrogated when the B cells are infected with Epstein–Barr virus, a ubiquitous γ1-herpesvirus that entails the strongest non-genetic risk factor for MS.

Keywords 
EAE · Autoimmune · Tolerance · B cells · Antigen presentation

Introduction

During fetal development, the immune system is instructed to react against foreign agents (non-self antigens) while ignoring components from the host’s body (self antigens). The thymus has a central role in this process, as T cells competent to react against self are eliminated from the repertoire via negative selection, while T cells recognizing non-self antigens presented by the host’s major histocompatibility complex (MHC) molecules are allowed to enter the repertoire via positive selection (Nossal 1991). Nevertheless, studies in laboratory animals (mice, rats, primates) revealed that T cells capable of inducing autoimmune-driven neuroinflammatory disease are present in the healthy immune repertoire, suggesting that these autoreactive specificities have escaped thymic (negative) selection (Ben-Nun et al. 1981; Meinl et al. 1997; Schluesener and Wekerle 1985; Villoslada et al. 2001). Using the well-validated experimental autoimmune encephalomyelitis (EAE) model in common marmosets (Callithrix jacchus), a small bodied Neotropical primate, we have explored how pathogenic T cells specific for the pathogenically relevant myelin antigen myelin oligodendrocyte glycoprotein (MOG) (Jagessar et al. 2008) are maintained inactive in healthy animals and how they are activated under conditions relevant to multiple sclerosis (MS), the human disease on which the EAE model has been projected. This short review gives a concise overview of these studies.

The EAE Model in Common Marmosets

EAE in common marmoset monkeys (Callithrix jacchus) is a validated animal model of the human autoimmune neuroinflammatory disease MS (’t Hart et al. 2015). The model has a high face validity for MS as it replicates essential clinical and pathological aspects of the human disease (’t Hart et al. 1998). Moreover, evidences from immunotherapy and mechanistic studies performed over the past two decades reveal a high construct validity, indicating that pathogenic mechanisms operating in the model are representative for the human disease (Kap et al. 2016). These features underscore

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the translational relevance of the model for research into pathogenic mechanisms as well as therapy development.

Recent work shows that the model is potentially useful for studies on the biological underpinning of factors that increase the risk of developing MS, such as infection with Epstein–Barr virus (EBV) (‘t Hart et al. 2013). EBV is a γ1-herpesvirus that infects human B lymphocytes via binding to complement C3d receptor (CD21) (Fingeroth et al. 1984). Importantly, the marmoset carries a natural infection with an EBV-related γ1-herpesvirus called CalHV3 that has comparable effects on the B cells (Cho et al. 2001).

After the discovery that B cell depletion via a monoclonal antibody (mAb) directed against the B lineage specific marker CD20 has a profound clinical effect in MS, the B cell has gained profound interest as a relevant target of therapy (Hauser et al. 2008). Newly recognized pathogenic functions of B cells beyond their traditional role, being production of autoantibodies that opsonize myelin, are cytokine production, the organization of ectopic lymphoid strotuctions within the central nervous system and antigen presentation to T cells (von Budingen et al. 2015). This short review will discuss data on the latter role of B cells obtained in the marmoset EAE model.

B Cells as Crucial Antigen-Presenting Cells in MS and Its Animal Model EAE

Marmosets immunized with myelin isolated from the brain of an MS patient, which was obtained via the Netherland’s brain bank (Amsterdam, Netherlands), developed an inflammatory demyelinating autoimmune disease that shows remarkable clinical and pathological similarities with MS (‘t Hart et al. 1998; Absinta et al. 2016). Our studies in mice and marmosets revealed that among the multitude of candidate myelin autoantigens, the quantitatively minor myelin component MOG has a central immunopathogenic role (Jagessar et al. 2008; Smith et al. 2005). In a marmoset EAE model elicited with recombinant human (rh) MOG, two peptides located in the Ig-like extracellular domain were found to contain immunodominant T cell epitopes, namely MOG14-36 (residues 24–36 identified as epitope for MHC class II/Caja-DRB4*W1201-restricted CD4+ T cells (Brok et al. 2000)) and MOG34-56 (residues 40–48 identified as epitope for MHC class I/Caja-E-restricted CD8+CD56+ T cells (Jagessar et al. 2012b)). The two peptides elicited distinct pathogenic mechanisms, which to some extent represent the relapsing–remitting and progressive phases of MS, respectively (‘t Hart et al. 2011).

Our studies revealed a central pathogenic role of B cells in marmoset EAE as late-stage depletion (from post-immunization day 21 onward) with a clonal variant of the clinically tested anti-CD20 mAb ofatumumab-suppressed clinical EAE development in marmosets sensitized against the full-length rhMOG protein (Kap et al. 2010, 2011) or the immunodominant peptide MOG34-56 (Jagessar et al. 2012a). A model induced with the MOG34-56 peptide is of particular interest, as the expression of MS-like pathology and clinical signs in this model is driven by the interaction of B cells with cytotoxic CD8+ CD56+ effector memory T cells having IL-17A as cytokine signature (‘t Hart et al. 2017). Notably, MS pathology in the MOG34-56-induced model developed in the absence of antibodies capable of binding myelin, as these were not detectable in the circulation (Jagessar et al. 2015).

The promising clinical effect of anti-CD20 mAbs sparked tests of other B cell depletion strategies. It was found in the rhMOG-induced marmoset EAE model that treatment with antibodies capturing cytokines that mediate survival and differentiation of B cells, i.e., BLyS and APRIL, did lead to B cell depletion from the peripheral blood, but exerted only a marginal effect on the expression of EAE symptoms or pathology (Jagessar et al. 2012a). Atacicept is a chimeric construct consisting of the Fc tail of human IgG and the joint receptor of BLyS and APRIL. This study in the EAE model essentially replicated the failure of atacicept in relapsing MS clinical trials (Kappos et al. 2014). The reason for this discrepant clinical effect was examined in the marmoset EAE model, revealing that treatment with anti-BLyS and anti-APRIL antibodies failed to deplete CalHV3 (+infected B cells) from the lymphoid system, while treatment with anti-CD20 mAb depleted also this subset (Jagessar et al. 2013). These findings led us to hypothesize that a critical pathogenic effect of B cells is mediated by a small-sized subset of CalHV3-infected B cells (<0.05% of all B cells) (Khan et al. 1996), which present a key pathogenic peptide (MOG34-56) to autoaggressive T cells. This finding might give a plausible mechanistic explanation for the close association of EBV infection and MS risk (‘t Hart et al. 2017).

Cathepsin G as a Leading Protease in the Processing of MOG in (EBV-Infected) B Cells

We observed that immunization with rhMOG in the mineral oil IFA induced MS-like pathology in three non-human species, indicating that autoreactive T cells present in their naïve repertoire can be activated without the normally requisite co-stimulation by danger signals (Haanstra et al. 2013). Immune profiling of this atypical EAE model in marmosets revealed that T cell and antibody reactivity against several MOG peptides, i.e., MOG14-36, MOG24-46 and MOG54-76, could be detected while reactivity of blood mononuclear cells and serum with the MOG34-56 peptide was conspicuously absent (Jagessar et al. 2015). However, the model induced with MOG34-56/IFA demonstrates that T and B cells reactive with MOG34-56 peptide are present in the marmoset’s repertoire (Jagessar et al. 2010). Hence, we hypothesized that the peptide might be
destroyed during processing in antigen-presenting cell (APC), being (CalHV3-infected) CD20⁺ B cells, probably as an effective strategy to avoid activation of autoreactive T cells that have escaped negative selection in the thymus (Manoury et al. 2002).

Experiments testing the degradation of rhMOG and MOG34-56 in lysates of non-human primate CD20⁺ spleen cells in the presence of protease inhibitors revealed that the degradation of both proteins is inhibited by addition of the cathepsin G antagonist CMK (Jagessar et al. 2016), indicating that cathepsin G may lead to autoantigen degradation, as was observed earlier by Burster et al. (2004) for another myelin antigen (MBP). Cathepsin G is a serine protease that has gained multi-specificity during mammalian evolution due to mutation of specificity-determining residues at positions 189 and 226 (Raymond et al. 2010). While cathepsin G of mice (Ser189/Ala226) has narrow chymotrypsin-like specificity for the native MOG34-56 peptide in two preparations of human B cells, respectively, naïve B cells stimulated with CpG oligonucleotides (Jagessar et al. 2010, 2012b; Kap et al. 2008). The epitope seems to be a hot-spot of proteolytic activity as, besides the above-discussed cleavage by cathepsin G, it may also be a target of TSSP, a thymus-specific serine protease involved in the processing of antigen expressed by thymic epithelial cells. TSSP is a proline-specific dipeptidase that was found to abort negative thymic selection of MOG34-56-specific encephalitogenic T cells in C57BL/6 mice by destructive processing of the MOG40-48 epitope in thymic epithelial cells (Serr et al. 2017). It is tempting to speculate that the destructive processing of the same epitope by cathepsin G in B cells may function as a peripheral backup system for preventing activation of encephalitogenic T cells that have escaped thymic selection.

The highlighted (yellow) sequence (40YRPPFSRNVHLRNGKD) (Jagessar et al. 2016). It has been documented that B cells are poorly capable of antigen cross-presentation, unless they are infected with a γ1-herpesvirus (Jagessar et al. 2012b) or stimulated via TLR9 with CpG (Jiang et al. 2011).

Among the multiple changes that EBV infection causes in B cells, two processes seem to be of particular importance, namely the induction of peptidyl-arginine deiminase (PAD) activity and the activation of autophagy (Jagessar et al. 2016; Morandi et al. 2017). The importance of increased PAD activity is that peptide citrullination, altering antigen processing (Jagessar et al. 2016), can take place within EBV-infected B cells (Ireland and Unanue 2011). The induction of autophagy may be of particular interest, as epitope loading of MHC-E molecules seems not to occur in the endoplasmatic reticulum, where classical class I molecules are loaded, but in autophagosomes (Camilli et al. 2016).

These considerations prompted a next set of experiments in which we tested proteolytic degradation of native and citrullinated MOG34-56 peptide in two preparations of human B cells, and the native MOG34-56 epitope was abrogated when the Arg residues at positions 41 and 46 (underlined) were replaced by citrulline, despite the presence of at least three additional potential cleavage sites (bolded): GMEVGWYRPPPSRNVHLRNYRNGKD (Jagessar et al. 2016). The central question arising from the in vivo experiments in the marmoset EAE model has been: why does infection of B cells with EBV enhance their APC function? A critical finding has been that the MOG40-48 epitope is cross-presented via Caja-E to the CD8⁺ CD56⁺ CTL (Jagessar et al. 2012b).

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A Novel Pathogenic Role of EBV-Infected B Cell in the Marmoset EAE Model

A study in the mouse EAE model revealed that the Arg⁴¹ and Arg⁴⁶ residues are essential contact points for binding of the epitope to the antigen receptor of encephalitogenic CD4⁺ T cells and that citrullination at these positions abrogates T cell recognition (Carrillo-Vico et al. 2010). The obvious paradox created by this observation, when translated to the MOG34-56/IFA model in marmosets, is that protection of the peptide against destructive processing in B cells by cathepsin G requires citrullination of at least the Arg⁴⁶ residue, while this modification may abrogate T cell recognition. We
asked therefore whether there might be another pathogenic mechanism at work in the model.

To test this possibility, we used a version of the peptide that could be visualized during and after the incubation with B cells via tagging a fluorochrome. This was achieved by coupling propargylglycine as a bioorthogonal handle to a slightly elongated variant of the MOG34-56 peptide, i.e., 31–55. Figure 1 shows a representative example of non-citrullinated MOG peptide incubated for 48 h with human EBV-infected B cells. Clearly, the peptide is not degraded, but seems to be extruded in an aggregated form. Concomitantly, LC3 expression is strongly increased. These features may be explained by a recently documented novel feature of citrullinated MOG peptide, namely the capacity to form toxic amyloid-like aggregates (Araman et al. 2019).

Conclusion

Despite decades of intensive research, scientists have only superficial understanding of the critical processes in MS pathogenesis. Valuable insights can be gained from immunotherapy studies and by investigating the biological underpinning of MS risk factors, as these are very likely connected to rate-limiting steps in the disease process. The recognition that infection with EBV substantially enhances the risk of developing MS in genetically prone individuals (Ascherio and Munger 2015) converges with the observation in immunotherapy trials using anti-CD20 antibodies that the primary targets of the virus in the immune system, i.e. B cells, have a central pathogenic role in the disease (Barun and Bar-Or 2012).

Our research in the well-validated and translationally relevant marmoset EAE model revealed a central role of B cells in the control of autoimmunity against MOG, which seems to be exerted via destructive processing of the CD8 T cell epitope MOG40-48 by cathepsin G. This essentially tolerogenic mechanism is abolished when the B cells are infected with EBV. Analysis of the underlying mechanism revealed that the infection induces a post-translational modification in the epitope, i.e., replacement of Arg46 by citrulline, which enables association of the EAE inducing MOG34-56 peptide with autophagosomes. We hypothesized that via this route, the vulnerable MOG40-48 epitope may be protected against fast endolysosomal degradation by cathepsin G and that its loading onto Caja-E molecules may be promoted (‘t Hart et al. 2016).

Summarizing, we posit an important tolerogenic function of cathepsin G in the marmoset EAE model, namely to prevent the B cell-mediated activation of hazardous T cells by destroying their MOG40–48 autoantigen epitope during processing. Infection of the B lymphocytes with EBV induces cellular mechanisms, citrullination and autophagy, which protect the epitope against fast degradation, thereby enabling presentation to T cells. It would be highly interesting to test whether this principle can be extended to other autoantigens critical in other autoimmune diseases.

Fig. 1 Feeding MOG31-55 peptide to EBV-infected human B cells boosts LC3 expression. Human EBV-infected B cells were incubated for 48 h with the indicated doses MOG31-55. The peptide was labeled with the bioorthogonally handle Pg to enable its visualization via click chemistry (Araman et al. 2019). After the incubation, the cells were washed and subsequently spun onto microscopy slides (28g). The slides were processed for immunostaining for LC3 as described (Araman et al. 2019). Colors: blue = cell nuclei; green = autophagosomes; purple = the MOG peptide.
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