Autoimmune uveitis: study of treatment therapies

Uveíte autoimune: estudo de terapias para tratamento

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ABSTRACT

Experimental autoimmune uveitis is an organ-specific T-cell mediated autoimmune disease characterized by inflammation and consequent destruction of the neural retina and adjacent tissues. Inflammation in experimental autoimmune uveitis may be induced in rodents by immunization with retinal antigens, such as interphotoreceptor retinoid-binding protein. We present a review of experimental studies that correlate primary immunobiological functions with this chronic disease and the possible use of molecules for the treatment of autoimmune uveitis.

Keywords: Uveitis/immunology; Disease models, animal; Antigens/immunology

RESUMO

A uveíte autoimune experimental é uma doença autoimune mediada por células T, órgão-específica e caracterizada por inflamação e subsequente destruição da retina neural e tecidos adjacentes. A inflamação na uveíte autoimune experimental pode ser induzida em roedores pela imunização com antígenos retinianos, tais como a proteína interfotorreceptora ligante de retinoide. Apresentamos aqui uma revisão de estudos experimentais que correlacionam as principais funções imunobiológicas com esta doença crônica e o possível uso de moléculas para o tratamento da uveíte autoimune.

Descritores: Uveíte/imunologia; Modelos animais de doenças; Antígenos/imunologia

INTRODUCTION

The immune system is a complex network involving cells and molecules compromised with immunological responses to pathogens, maintenance of self-tolerance, generation of specific memory and adaptation. This network, essentially pleiotropic, is controlled by high polymorphic loci with elevated adaptive values of traits, such as the major histocompatibility complex (MHC)[1], the components of the complement system[2], as well as genes that regulate the expression of variable regions of immunoglobulin[3] and the T-cell receptor (TCR)[4]. Moreover, the main immunobiological functions, such as antibody production, inflammatory reactivity, tolerance, resistance to infections and toxin actions are quantitative traits submitted to independent polygenic controls[5-6]. Therefore, all evolutionary processes are related to diversity, ensuring the survival of species, and two main genetic peculiarities play an essential role: polymorphism and polygenes. The triad of diversity, specificity and complexity represents the basic and fundamental aspects of immunobiological events ensuring the multidirectional protection of a genetically heterogeneous natural population.

Naturally occurring subliminal immune phenomenon intervening for maintenance of neutralization and/or equilibrium and ordered states of biologically active endogenous molecules must be frequent. Under variable and constant environmental pressures along life, the organism expresses several molecular targets that could be susceptible to a series of autoimmune episodes that are surmountable due to homeostasis. However, unbalances in the relations between cells and molecules with expressions increased of multifunctional proteins, such as IL-6, Hsp, and

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TNF, can trigger chronic, cumulative and irreversible processes of autoimmunity that are influenced by a combination of genetic and environmental factors[7].

EXPERIMENTAL AUTOIMMUNE UVEITIS

Experimental autoimmune uveitis (EAU) is an organ specific T-cell mediated disease that targets the posterior pole of the eye and a well-characterized model that is valuable for the study of human idiopathic uveitis. EAU may be induced in susceptible primates and rodents after immunization with retinal self-antigens, such as interphotoreceptor retinoid-binding protein (IRBP) or S-antigen (arrestin), or by the adoptive transfer of T-cells specific for these antigens[8-10].

The model of EAU in mice contributed to the establishment of parameters for evaluation of possible therapies for posterior uveitis in humans[8]. Studies of genetic susceptibility and resistance to EAU[11], characterization of uveitogenic epitopes[12], and studies of tolerance in EAU by immune deviation associated with anterior chamber[13-14] or systems of oral tolerance[15-16] obtained success when this model was employed.

It must be emphasized that the currently used experimental model, disseminated by the developed countries, is limited to one or two genetically homogeneous mouse isogenic lines were for a given character the phenotypic variance (VP) correspond to the environmental variance (VE). Bear in mind that an inbred line is obtained by sibling intensive crossing during successive generations. The genetic partition is never evidenced, and the results do not allow the establishment of real biological relevance of innate and/or acquired immune factors. The eventual correlation amongst distinct immunobiological parameters and the resistance or susceptibility to autoimmune progression is merely fortuitous.

In order to introduce a new concept for investigating autoimmune uveitis in mice, and aiming to determine the influence of genetic backgrounds and factors (VG) intervening in the innate and/or acquired immune functions in the development of EAU and to establish the possible association of specific anti-IRBP isotype profiles in the susceptibility to the disease, some studies were carried out in genetically modified lines of mice selected high (H) or low (L) antibody responses and for maximal or minimal acute inflammatory responses [AIR_{MAX} and AIR_{MIN}, respectively]. These four non-inbred genetically selected lines of mice resulted in the convergent fixation of alleles affecting the high or low antibody or inflammatory responses regarding the acquired and/or innate immune compartments. This approach allows the study of the disease in populations selected for important immune response traits, but which are still heterogeneous with regard to the rest of their genome, thus better resembling the human population[17-18].

The EAU development and the anti-IRBP IgG1 and IgG2a antibody production, two IgG isotypes representatives of the Th2 and Th1 T helper lymphocyte series were investigated in these genetically modified mice and definitively proved that, unlike in inbred strains, anti-IRBP responses were not correlated to the susceptibility to the development of EAU. For both antigen-specific IgG1 and IgG2a, the analysis of variance corroborates the importance of multigenic factors regulating the adaptive responses to IRBP. Moreover, based on the distinct the major histocompatibility H–2 alleles of the four mouse lines and the similarities of the EAU scores, especially between the AIR_{MAX} and L_{III} strains, no specific MHC allele seems to be crucial for the development of the disease, as it is in inbred strains. It must be pointed out that the L_{III} mice are H–2^z, H_{III} are H–2^{o3}; the AIR_{MAX} are predominantly H–2^b and on AIR_{MIN} mice, H–2^d and H–2^k are the prevalent haplotypes.

Therefore, the genetic control of the immune characteristics during the autoimmune process in EAU is polygenic, since the interline variances were always higher than the intraline ones, and there were continuous distributions among individuals[19].

NEW THERAPIES IN EAU

The course of the EAU is characterized by vasculitis and granuloma formation in the neural retina, destruction of photoreceptor cells and blindness[8,10] caused by infiltrating lymphocytes and inflammatory cells. Migration of activated lymphocytes into the eye is facilitated by binding of surface proteins on these cells with adhesion molecules on the endothelium. Immunization of B10.A mice with IRBP induces expression of intercellular adhesion molecule-1 (ICAM-1) in the vascular endothelium of the ciliary body and retina, as well as the expression of lymphocyte function-associated antigen-1 (LFA-1) on inflammatory cells that enters the eye[20]. The observation that ocular inflammation was significantly decreased after administration of monoclonal antibodies (mAbs) against ICAM-1 and LFA-1[20] attributes an important role for these adhesion molecules in EAU. In addition, an *in vitro* study confirmed that lymphocyte adhesion and transmigration across monolayers of IFN-γ activated retinal pigmented epithelial (RPE) cells are inhibited by mAbs against the very late antigen-4 (VLA-4) and vascular cell adhesion molecule-1 (VCAM-1) in a rat model of uveitis[21]. The expression of VCAM-1 in blood vessels of the retina during the development of

EAU also seems to be involved in lymphocyte migration into the eye[21-22].

In 2005, our group demonstrated that treatment with an α4 active peptide inhibitor (α4-api), which targets the α4 integrin of the VLA-4 adhesion molecule, had a significant ameliorating effect on EAU[23]. These results indicate that α4 integrins are indeed essential for the recruitment of lymphocytes into the eye and that blockade of integrin-ligand interactions may be effective in preventing the entry of uveitogenic cells.

Other findings showed that the administration of anti-LFA-1α antibody suppressed IRBP-induced EAU in C57Bl/6 mice by blocking the activation of uveitogenic T-cells and trafficking of autoreactive activated T-cells into the inflammatory site[24].

Clinical ocular pathology can also be prevented by the administration of recombinant Galectin-1 (rGal-1) either early or late during the course of EAU. Galectin-1 is a member of a highly conserved protein family[25], expressed at sites of T-cell activation and immune privilege[26-27], and has the potential to regulate inflammatory responses[28-34]. The treatment with rGal-1 in IRBP-induced EAU in B10.RIII mice resulted in significant amelioration of ocular inflammation. In these animals, delayed-type hypersensitivity and cellular proliferation decreased and the levels of T-regulatory cytokines, TGF-β and IL-10, increased by treatment with rGal-1, moreover, the IFN-γ levels was decreased (Figure 1). We observed that the GATA-3 transcription factor, involved in gene transcription of Th2-cytokines, was increased[35]. These results showed that manipulating the immune system to upregulate Th2- and T-regulatory cytokine may prevent inflammation and EAU development, as others studies had already demonstrated[36-39]. Other study reported that anti-retinal Gal-1 antibodies are present in serum from patients with uveitis, which suggests that these autoantibodies recognize retinal structures and play a role in the progression of ocular disease[40].

Recently, various reports clearly showed that some molecules, such as LX211 (voclosporin)[41], Fingolimod (FTY720)[42], anti-IL-17[43], alpha-melanocyte stimulating hormone (alpha-MSH)[44], were effective in suppressing autoimmune uveitis.

In this context, our group studied the efficiency of the viperidae *Lachesis muta* snake venom protein (LMVp) in the suppression of EAU and the mechanisms involved in the regulation of the disease. LMVp was initially described as strong suppressor of antibody production to sheep erythrocytes[45]. In a series of experiments, it was possible to demonstrate *in vivo* the suppressive effect of LMVp administered before immunization.

Preliminary results in the isogenic B10.RIII mice demonstrated, in a striking manner, that the treatment

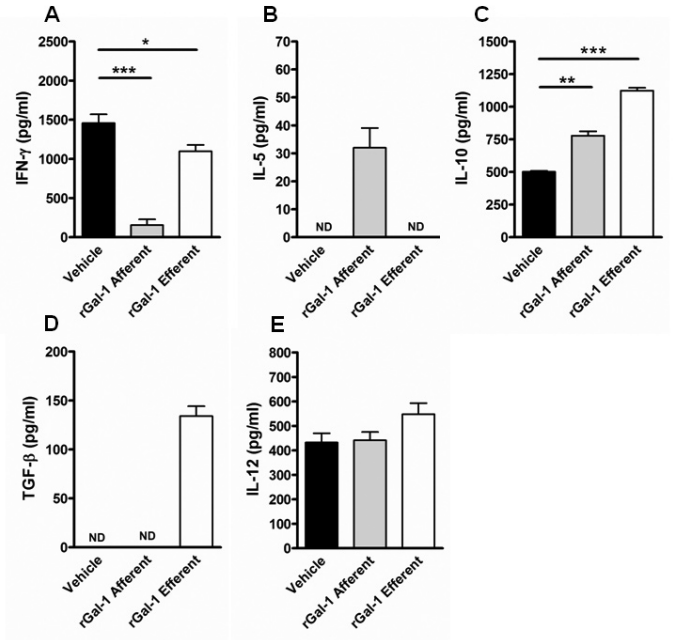

Figure 1. Treatment with rGal-1 at early or late phases of EAU turns the autoimmune response into non-pathogenic Th2 and Th3 regulatory cytokine profiles. (A-E) Draining lymph nodes cells from rGal-1-treated or control mice were harvested at day 21 and stimulated *in vitro* with 30 μg/ml IRBP. After 48 hours, IFN-γ (A), IL-5 (B), IL-10 (C), TGF-β (D) and IL-12 (E) levels were determined in culture supernatants by ELISA. Results are expressed as mean pg/ml ± standard deviation.
*p < 0.05; ** p < 0.01; *** p < 0.0001.
Source: Toscano MA et al.[35]

with the LMVp 72 hours before EAU induction abolished the development of uveitis in these animals; retinal structures were maintained and substantial reduction of leukocyte infiltration (Figure 2). However, the treatment with LMVp was not able to inhibit the specific-IRBP T-cell activation/proliferation. A decrease in the B-cell population (B220+) was observed in the LMVp-treated animals and suppression of both anti-IRBP IgG1 and IgG2a specific antibodies. These results suggest that the modulation of B-cell differentiation might inhibit a Th1-mediated disease, revealing the important participation of the humoral response in the EAU[46].

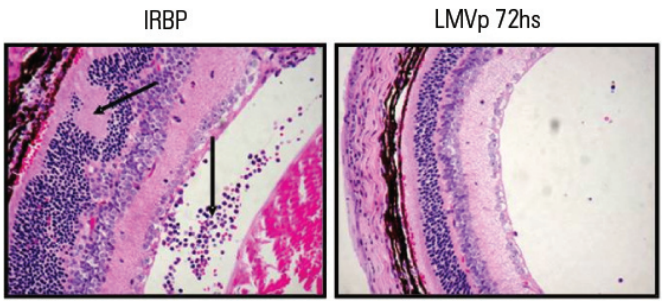

Figure 2. LMVp suppresses the clinical signs of actively induced EAU. B10.RIII mice were immunized with 100 μg of IRBP on day 0 and treated with 100 μg i.p. of LMVp 72 hours before immunization. Eyes were collected for histopathology 21 days after immunization. EAU scores were assigned on a scale from 0 to 4, according to the extent of inflammation and tissue damage.
Source: Commodaro et al.[46]

In the above mentioned results, it was evidenced the significant impact of environmental factors prevailing during this autoimmunity process. As recently hypothesized, the immunological history of an individual is unique and irreversible being cumulative, that is, the continued aggravation of an autoimmune process results in inability of tissue regeneration by affected systems[7]. In this context, the use of α4 inhibitors, rGal-1 and LMVp may contribute as an alternative therapeutic approach in the control of autoimmune ocular diseases.

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