Pharmacological Reports 66 (2014) 192–197

Contents lists available at ScienceDirect

Pharmacological Reports

journal homepage: www.elsevier.com/locate/pharep

Review article

Skin-induced tolerance as a new needle free therapeutic strategy

Marian Szczepanik

Department of Medical Biology, Jagiellonian University College of Medicine, Kraków, Poland

ARTICLE INFO

Article history:
Received 25 March 2013
Received in revised form 26 August 2013
Accepted 12 September 2013
Available online 4 March 2014

Keywords:
Contact hypersensitivity
Autoimmunity
Graft rejection
Skin-induced suppression

ABSTRACT

This article summarizes current knowledge about a new subject called "skin induced tolerance". Suppression is induced *via* epicutaneous (EC) immunization with a protein antigen and is described in Th1, Tc1 and NK mediated contact hypersensitivity (CHS) reactions. The subject of skin-induced suppression is also described in the regulation of experimental models of autoimmune diseases like experimental autoimmune encephalomyelitis (EAE), collagen induced arthritis (CIA) and inflammatory bowel disease (IBD) and finally in an animal model of graft rejection. The potential clinical use of this approach to regulate human diseases is also discussed.

Contents

Introduction

Both the skin and mucosa are constantly exposed to numerous antigens and play a crucial role in protecting the body from various pathogens present in the external world. While development of immune responses to pathogens is of vital importance to the macroorganism, response to innocuous antigens is, at best, not helpful and often leads to harmful allergy. It is well known that immunization with an antigen *via* the digestive tract or nasal mucosa leads both to a local immune response and a state of profound immunosuppression in the periphery [31,50]. Mucosal tolerance seems to play an important role in avoiding the development of immune responses to non-harmful antigens.

It was already shown that certain types of regulatory T cells are preferentially induced in mucosa to maintain tolerance [47]. Moreover, it is believed that a special mucosal milieu may create tolerogenic dendritic cells that induce different populations of regulatory cells.

Although the skin is considered an organ where immune responses are easily induced [3], little attention has been given to skin induced tolerance [37]. Because skin and mucosa play similar function in our bodies (*i.e.* as a barrier to external pathogens) it is

Abbreviations: CD, Crohn's disease; CHS, contact hypersensitivity; CIA, collagen-induced arthritis; CNS, central nervous system; COLL II, bovine type II collagen; DNFB, dinitrofluorobenzene; DNP, 2,4-dinitrophenol; DTH, delayed type hypersensitivity; EAE, experimental autoimmune encephalomyelitis; EC, epicutaneous; HAI, histological activity index; IBD, inflammatory bowel disease; IFN-γ, interferon gamma; IL, interleukin; KLH, keyhole limpet hemocyanin; MBP, myelin basic protein; MOG, myelin oligodendrocyte glycoprotein; MPO, myeloperoxidase; MRI, magnetic resonance imaging; OVA, ovalbumin; OX, oxazolone; PLP, myelin proteolipid protein; RA, rheumatoid arthritis; TGF-β, transforming growth factor beta; Th1, T helper 1; Th2, T helper 2; Th17, T helper 17; TNBS, trinitrobenzene sulfonic acid; TNP-Cl, 2,4,6-trinitrobenzene sulfonic acid; TNP-Ig, TNP conjugated mouse immunoglobulins; Treg, T regulatory cell; Ts, T suppressor cell; UC, ulcerative colitis.

E-mail address: mmszczep@cyf-kr.edu.pl.

http://dx.doi.org/10.1016/j.pharep.2013.09.001

possible that epicutaneous (EC) application of antigen, apart from inducing a strong immune response, may also induce suppression in the periphery. It was shown previously that EC immunization with the protein antigen ovalbumin (OVA) resulted in allergic dermatitis accompanied with the induction of IL-4 and IL-13 synthesis [43,45]. Further, it was found that EC immunization with OVA induced a Th2 mediated model of asthma in mice [15]. Finally, it was demonstrated that local administration of IFN-γ during the sensitization phase of protein antigen immunization suppresses development of Th2-mediated atopic dermatitis in mice [46].

These complex studies on EC immunization suggested to us that similar to mucosal immunization, application of protein antigens on the skin may also induce T lymphocytes producing anti-inflammatory cytokines that could inhibit Th1/Th17-mediated immune responses causing their suppression.

Epicutaneous immunization with hapten-conjugated protein antigen inhibits contact hypersensitivity in mice

Contact hypersensitivity (CHS) is a classical form of *in vivo* T cell-mediated immunity induced by topical skin immunization with haptens. CHS responses can be mediated by either $CD4^+$ Th1, MHC class II-restricted lymphocytes locally producing IFN-γ to recruit a typical inflammatory infiltrate [18], or by $CD8^+$ MHC class I-restricted Tc1 cells that can similarly release IFN-γ, but predominately mediate cytotoxic damage to local skin cells such as keratinocytes [26]. Finally, the discovery by von Adrian's group proving that NK cells may act as effector cells in CHS in mice was a breakthrough in research on the mechanisms involved in CHS response [29]. Further our studies demonstrated that the NK cells able to adoptively transfer CHS response belong to CXCR6-expressing subset [32]. In contrast to Th1- and Tc1-mediated CS, NK cell-mediated CHS occurs independently of B-1 or NKT lymphocytes but is IFN-γ, IL-12 and IFN-α dependent [23].

CHS like all other immune responses are under strict control of regulatory mechanisms. It was shown previously that this type of immune response can be negatively regulated by T suppressor (Ts)/T regulatory (Treg) cells induced *via* intravenous injection [4] or oral deposition of high dose of antigen [10]. Our research showed for the first time that apart from intravenous and oral antigen administration also EC deposition of protein antigen inhibits CHS.

Work on skin-induced suppression was inspired by previous findings showing that EC immunization with protein antigen induces production of IL-4 and IL-13 that can potentially inhibit Th1-mediated immune response.

To test if EC immunization with protein antigen could inhibit cell-mediated immune responses we have developed the procedure presented below. The efficacy of EC induced suppression was tested in three different models of CHS.

First, we tested skin-induced suppression in Th1-mediated CHS. Mice were exposed to hapten-conjugated mouse immunoglobulins, TNP-Ig on days "0" and "+4" spread over the gauze patch. Then, on day "+7" patches were removed and animals were sensitized with TNP-Cl and tested for CHS four days later. This simple experiment proved that EC immunization with protein antigen results in strong decrease of Th1-mediated CHS [38]. To avoid cumbersome and inconvenient patch method we developed another technique based on daily application of neutral cosmetic Nivea cream containing different protein antigens as additives on the shaved skin [34]. Similarly to patch method EC immunization with an antigen emulsified in cream caused strong inhibition of CHS.

Further experiments in TNP-Cl model showed that skin-induced suppression is dose dependent and optimal dose of antigen that induces this phenomenon is between 30 and 100 μg/animal [38]. Additionally we found that EC induced suppression lasts for about four weeks. Using both "transfer out" and "transfer in" protocols we showed that skin-induced suppression is transferable and that EC induced suppressor cells were very potent as they were able to significantly inhibit CHS effector cells when transferred into naïve recipients at a ratio of 1–10 [38]. Then, negative selection experiments with proper mAb and complement, cell sorting experiments and use of NKT cell-deficient $J\alpha18^{-/-}$ mice revealed that EC induced suppressor cells belong to the rare population of $TCR\alpha\beta^+$ $CD4^+$ $CD8^+$ cells. $CD4^+$ $CD8^+$ T cells in naïve mice are less than 1% of peripheral lymphocytes and their origin is unclear. These cells may represent $CD4^+$ $CD8^+$ T cells that have prematurely escaped from the thymus or $CD4^+$ $CD8^-$ T cells that re-express CD8 after activation or exposure to cytokines *e.g.* IL-4, or are present due to prior "natural" suppressive events. In our case we believe that $CD4^+$ $CD8^+$ T cells with regulatory activity originate from prematurely escaped $CD4^+$ T cells that acquire CD8 co-receptor. Our hypothesis is based on the observed increase in $CD4^+$ $CD8^+$ T cells in lymph nodes 7 days post EC immunization, and their subsequent decrease to the level observed in naïve mice within a week. These data can also suggest that these immature $CD4^+$ $CD8^+$ suppressor cells then may become single positive $CD4^+$ T lymphocytes [38].

Experiments with various non-cross reacting antigens such as TNP, oxazolone (OX), ovalbumin (OVA), keyhole limpet hemocyanin (KLH), elastin, collagen and keratin showed that the final mediation of suppressor T cell activity is antigen non-specific [34,38].

The association of antigen non-specific suppression with inhibitory cytokines led us to test if a specific cytokine milieu is required for induction of Ts cells *via* EC immunization, as observed in some other systems [33]. To determine whether a specific cytokine milieu is required to induce suppressor cells *via* EC immunization, mice were treated with TNP-Ig alone or with TNP-Ig plus anti-cytokine antibodies: anti-IL-4, anti-IL-10 or anti-TGF-β or control antibody on the skin prior to induction of CHS. All of the tested anti-cytokine mAbs significantly reduced suppression induced *via* EC immunization with TNP-Ig, suggesting that anti-inflammatory cytokines are indeed involved in the induction of suppressor cells [38]. Finally, *in vitro* studies with cytokine neutralizing antibodies showed that TGF-β but not IL-4 or IL-10 plays a crucial role in effector phase of skin-induced suppression [38].

To test whether EC immunization with an antigen can also inhibit CD8 dependent immune responses, we employed Tc1-mediated CHS to dinitrofluorobenzene (DNFB). This study showed that similarly to skin-induced suppression of Th1-dependent CHS, EC induced inhibition of Tc1-dependent CHS is dose dependent and optimal dose of antigen that gives suppression is between 1 and 100 μg/animal [52]. Epicutaneously induced suppression lasted at least three weeks since patch removal. Adoptive cell transfer experiments showed that skin-induced suppression can be transferred with lymphoid cells isolated from previously patched donors [25,52]. Negative selection experiments with proper mAb and complement, flow cytometry, MACS cell sorting experiments and use of $TCR\delta^{-/-}$, $\beta_2m^{-/-}$ and $CD1d^{-/-}$ mice showed that EC induced suppressor cells belong to the population $TCR\alpha\beta^+$ $CD4^+$ $CD25^+$ $FoxP3^+$ T regulatory (Treg) cells [25]. Additionally, our adoptive transfer experiments showed that $CD4^+$ $CD25^+$ Treg cells isolated from mice EC immunized with DNP-BSA could potently suppress CHS response by effector cells at a ratio of 1–35 (2×10^6 Treg cells *vs.* 7×10^7 effector cells) [25]. This was similar to our previous findings that very low numbers of skin-induced $CD4^+$ $CD8^+$ suppressor cells, such as 2×10^3 per mouse, were able to inhibit the effector function of 7×10^7 4-day TNP-Cl immune cells *in vivo* [38]. Further, our *in vitro* experiments showed that EC