

LOCAL BUT NOT SYSTEMIC ADMINISTRATION OF IFN- γ DURING THE SENSITIZATION PHASE OF PROTEIN ANTIGEN IMMUNIZATION SUPPRESS Th2 DEVELOPMENT IN A MURINE MODEL OF ATOPIC DERMATITIS

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Biphasic Th1/Th2 development plays a central role in the pathogenesis of atopic dermatitis. In the sensitization phase after protein antigen exposure, an immune response polarized toward Th2 differentiation, which is due to the hosts' genetic proneness to the disease, initiates the skin lesions. Th1/Th2 antagonism is a potential mechanism that could be manipulated to suppress the initial Th2 deviation. IL-12 is the key cytokine for Th1 differentiation. Interferon γ (IFN- γ) can assist Th1 development through several mechanisms and suppress Th2 differentiation. We took advantage of a recently developed murine model of atopic dermatitis elicited by epicutaneous sensitization with protein antigen through patch application to examine the effects of different routes of IFN- γ administration on Th1/Th2 differentiation during the sensitization phase of antigen exposure. Our data showed that systemic administration of IFN- γ during the sensitization phase could not promote serum levels of specific IgG_{2a}. However, local administration (intradermal injection or patch application) of IFN- γ during the sensitization phase could promote serum levels of specific IgG_{2a} and suppress serum levels of specific IgE. Moreover, pretreatment of local IFN- γ with protein antigen has a long-term modulatory effect on serum levels of specific IgG_{2a} and IgE after repeated antigen immunization. Our results demonstrate that local but not systemic administration of IFN- γ during the sensitization phase of protein antigen immunization could suppress the Th2 deviation in this murine model of atopic dermatitis. Thus, this may represent a novel strategy for the treatment and prevention of atopic dermatitis.

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It is not disputed that the single determining cytokine for Th1 differentiation is interleukin (IL)-12. IL-12 is produced by dendritic cells, neutrophils, and macrophages in response to microorganisms and other stimuli.¹ Although mice with disrupted IFN- γ and IFN- γ receptor genes show qualitatively normal Th1 differentiation, IFN- γ could assist Th1 development through the following mechanisms.^{2,3} First, IFN- γ could enhance macrophage production of IL-12 in

response to pathogens, and thus, the Th1 response modality would be stabilized by a positive feedback loop.^{4,5} Second, IFN- γ could act directly on naïve T cells to promote IL-12 responsiveness for Th1 development.^{6,7} Third, IFN- γ could prevent early Th cells from premature commitment to the Th2 pathway by overriding the IL-4-induced inhibition of IL-12R β 2 expression.⁸ Moreover, IFN- γ has been demonstrated to suppress Th2 differentiation *in vivo* and *in vitro*.⁹

Atopic dermatitis (AD) is a chronic, relapsing inflammatory skin disease, which is characterized by extreme pruritus, typically distributed eczematous skin lesions, and a personal or family history of atopic diseases.¹⁰ Several theories have been proposed to understand the pathogenesis and etiology of AD, including an allergic theory, β -adrenergic blockade theory, S-6 desaturase enzyme deficiency, neurogenic inflammation, and autoimmunity.^{10,11} Despite these theories, the etiology and pathogenesis of AD are still not precisely understood. Nevertheless, it is generally agreed that an immunological dysregulation of

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biphasic Th1/Th2 activity plays a central role in the pathogenesis of AD.^{12,13} In the initial sensitization phase after protein antigen exposure, an immune response towards Th2 differentiation develops due to host's genetic proneness and to the site of antigen entry. This is reflected in high serum IgE levels, eosinophilia, and marked infiltration of acute skin lesions with T cells secreting IL-4, IL-5 and IL-13.^{14,15} However, with the progression of the skin lesions accompanied by the infiltration of eosinophils and macrophages occurring in chronic AD lesions, IL-12 expression increases and Th2 switches to Th1 immune responses.^{14–17}

We and those in Geha's group have developed a murine model of AD which shows that epicutaneous sensitization of protein antigen with patch application induces a predominant Th2 response with high IgE production and a dermatitis with AD characteristics.^{18,19} In the current study, we took advantage of this murine model to examine the effects of different routes of IFN- γ administration during the sensitization phase of protein antigen exposure on Th1/Th2 differentiation. Our results demonstrated that local but not systemic IFN- γ could modulate Th1/Th2 balance in this murine model of ADP.

RESULTS

Intraperitoneal administration of IFN- γ during the sensitization phase of OVA immunization has no effect on serum OVA-specific IgG_{2a} levels

Since it has been shown that systemic IFN- γ can promote in vivo Th1 responses, we first examined the effect of intraperitoneal (i.p.) administration of IFN- γ during the sensitization phase of OVA immunization on the development of Th1/Th2 differentiation. Serum levels of OVA-specific IgG_{2a} were chosen to indicate the in vivo OVA-specific Th1 response.²⁰ As shown in Figure 1, mice receiving i.p. IFN- γ during the sensitization phase of OVA immunization showed similar levels of OVA-specific IgG_{2a} in their serum after challenge when compared to control animals. No toxicity or crossreaction between OVA and IFN- γ were observed in mice receiving i.p. IFN- γ only without OVA immunization (data not shown). Thus, systemic administration of IFN- γ during the sensitization phase of OVA immunization could not promote the in vivo Th1 response in this model.

Local administration of IFN- γ during the sensitization phase of OVA immunization could modulate Th1/Th2 differentiation

For the second approach, we decided to administer IFN- γ locally. Two methods were chosen. One is

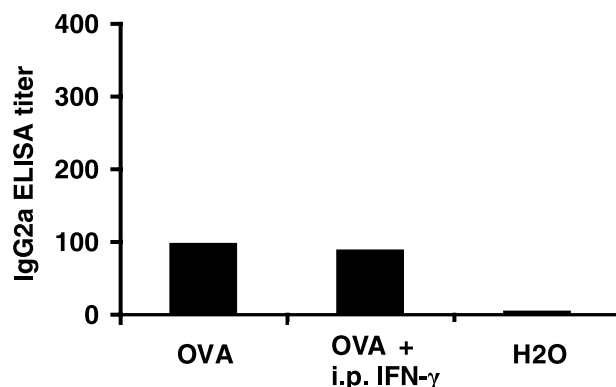


Figure 1. Intraperitoneal administration of IFN- γ during sensitization phase of OVA immunization has no effect on serum OVA-specific IgG_{2a} levels.

Two groups of mice, 8–10 mice per group, were immunized by epicutaneous patch application of OVA solution on days 1, 3, 5 of sensitization course. For intraperitoneal group, mice received 25 000 U IFN- γ each time via the i.p. route 2 h before patch application on days 1, 3, 5. One week after sensitization course, mice received challenge course of OVA-patch immunization without IFN- γ administration. Group of mice applied with H₂O patches served as control group. One week after the beginning of challenge course, mice were bled and sera were collected. Serum levels of OVA-specific IgG_{2a} were determined by ELISA. Geometric means of each group are presented. The results were from one representative experiment out of three performed.

intradermal injection (i.d.), which is a standard method to transfer the material into the skin, and the other is patch application (patch), which is the way we apply antigen epicutaneously in this animal model. Mice receiving i.d. IFN- γ during the sensitization phase of OVA immunization showed higher levels of OVA-specific IgG_{2a} in their serum after challenge when compared to the control group (Fig. 2). Interestingly, mice receiving patch application of IFN- γ also showed higher serum levels of OVA-specific IgG_{2a} when compared to control group, although their serum OVA-specific IgG_{2a} levels were lower than the i.d. IFN- γ group (Fig. 2). These data demonstrate that local administration of IFN- γ , either by intradermal injection or patch application, could promote in vivo OVA-specific Th1 responses. We next examined whether local administration of IFN- γ could suppress the in vivo Th2 response. Serum levels of OVA-specific IgE were chosen to indicate the in vivo Th2 response.²⁰ As shown in Figure 3, both groups of mice receiving i.d. or patch IFN- γ during the sensitization phase of OVA immunization showed lower levels of OVA-specific IgE in their serum after challenge when compared to the control group. Taken together, these results indicate that local administration of IFN- γ during the sensitization phase of protein antigen immunization could modulate Th1/Th2 differentiation in this animal model of AD.

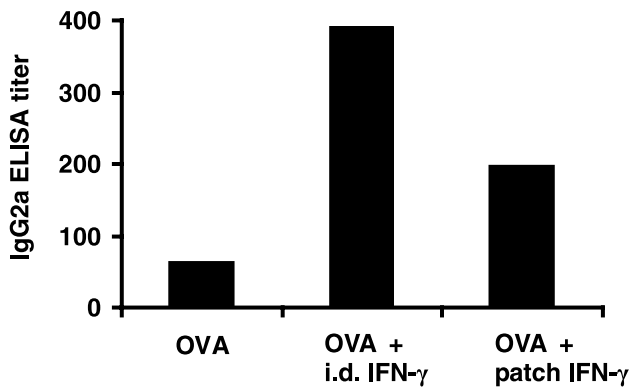


Figure 2. Local administration of IFN- γ during sensitization phase of OVA immunization could promote Th1 response.

Three groups of mice, 8–10 mice per group, were immunized by epicutaneous application of OVA solution on days 1, 3, 5 of sensitization course. For intradermal group, mice received 12 500 U IFN- γ each time via the i.d. route 15 min before patch application on days 1, 3, 5. For patch group, 12 500 U IFN- γ each time was added to patches on days 1, 3, 5. One week after sensitization course, all mice received challenge course of OVA-patch application without IFN- γ administration. One week after the beginning of challenge course, mice were bled and sera were collected. Serum levels of OVA specific IgG2a were determined by ELISA. Geometric means of each group are presented. The results were from one representative experiment out of three performed.

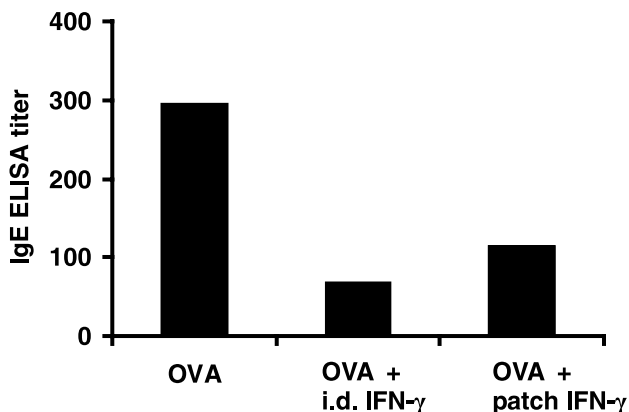


Figure 3. Local administration of IFN- γ during sensitization phase of OVA immunization could suppress Th2 response.

The procedures were similar to Figure 2. Serum levels of OVA-specific IgE were determined by ELISA. Geometric means of each group are presented. The results were from one representative experiment out of three performed.

Pretreatment of local IFN- γ with protein antigen has a long-term modulatory effect on Th1/Th2 balance after repeated antigen immunization

To determine whether local administration of IFN- γ with protein antigen has potential to vaccinate high-risk persons to prevent Th2 deviation from occurring after antigen exposure, we examined the effect of pretreatment of local IFN- γ with protein antigen on the Th1/Th2 balance after repeated antigen immunization in this animal model. All mice were divided into five groups. One group of mice was pretreated with a

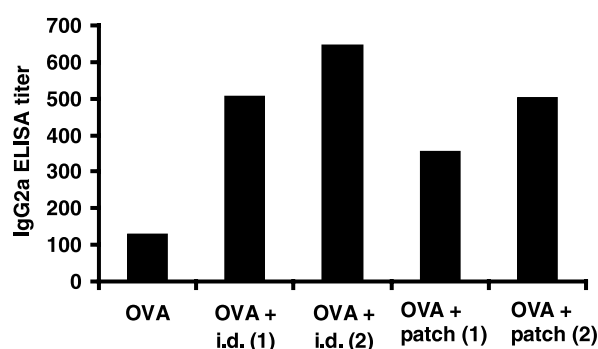
one-immunization course of i.d IFN- γ with protein antigen and another group received the same, except of patch IFN- γ with protein antigen. A third group of mice was pretreated with a two-immunization course of i.d. IFN- γ with protein antigen and a fourth got the same, except of patch IFN- γ with protein antigen. A fifth group of mice not receiving pretreatment served as control group. After pretreatment, all mice received OVA immunization by patch application for three courses in 2-week intervals. One week after the beginning of the third immunization course, mice were bled and the serum levels of OVA-specific IgG_{2a} and IgE were measured. Figure 4 shows that mice receiving pretreatment of i.d. or patch IFN- γ with OVA for one immunization course showed increased serum levels of OVA-specific IgG_{2a} and decreased serum levels of OVA-specific IgE, when compared to the control group of mice. Mice receiving pretreatment of i.d. or patch IFN- γ with OVA for two courses showed a further enhancement or suppression in their serum levels of OVA-specific IgG_{2a} and IgE, respectively. Thus, pretreatment of local IFN- γ with protein antigen had a long-term modulatory effect on Th1/Th2 balance after repeated antigen immunization in this murine model.

DISCUSSION

Although many different factors influence Th differentiation, the most important is the cytokine environment.²¹ IL-12 and IFN- γ are thought to be major cytokines for promoting Th1 differentiation. Although the presence of IL-12 during priming directly augments Th1 development, IL-12 can exacerbate rather than suppress Th2-dependent pathology in the absence of endogenous IFN- γ .²² In contrast, IFN- γ can suppress Th2 differentiation and has been demonstrated to be effective in the suppression of a Th2-predominant murine model of asthma.²³ Moreover, a similar condition seems to operate in the Th1-predominant immune response. It was reported that the contact hypersensitivity (CHS) response of IL-4 knock-out mice didn't differ from wildtype mice, whereas IL-10 knock-out mice mounted an exaggerated CHS response.²⁴ These are the reasons why we chose IFN- γ , but not IL-12, to test the possibility of suppression of Th2 deviation in a murine model of AD.

Although knock-out mice have been used to assess the role of IFN- γ in a murine model of AD, the knock-out mice system was unable to dissect the effect of the sensitization or elicitation phase.²⁵ Transgenic mice expression of IFN- γ in the epidermis exhibited a spontaneously developing cutaneous inflammation with an eczematous character.²⁶ The epidermis of these

A.



B.

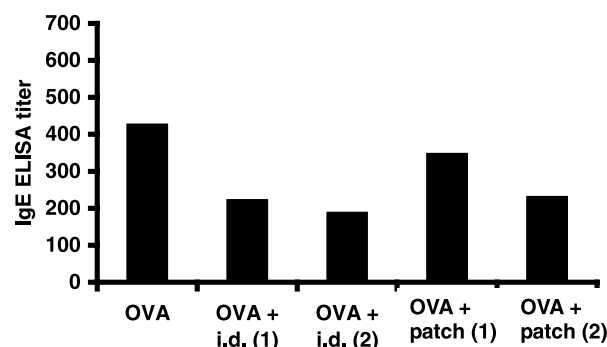


Figure 4. Pretreatment of local IFN- γ with protein antigen has long-term modulatory effect on Th1/Th2 balance after repeated antigen immunization.

For pretreatment, two groups of mice were immunized by epicutaneous OVA-patch application for one immunization course [i.d. (1) and patch (1)]; two group of mice were immunized by two immunization courses [i.d. (2) and patch (2)]; and OVA group received no pretreatment. Mice of i.d. or patch groups received intradermal or patch-applied IFN- γ administration during the pretreatment similar to Figure 2. After pretreatment, all mice received three successive immunization courses by OVA-patch application in a two week intervals without administration of IFN- γ . One week after the beginning of the third immunization course, mice were bled and sera were collected. Serum levels of OVA-specific IgG2a (A) and IgE (B) were determined by ELISA. Geometric means of each group are presented. The results were from one representative experiment out of two performed.

transgenic mice was devoid of Langerhan's cells (LC) and the number of LCs in their regional lymph nodes was increased. These transgenic mice also showed an increased contact hypersensitivity reaction.²⁶ Moreover, intradermal injection of IFN- γ into human volunteers induced transient skin inflammation with dermal perivascular lymphohistiocytic infiltrates, and a marked depletion of LCs in epidermis.²⁷ These observations strongly imply that in addition to its well-known effect on the effector phase of cutaneous immune responses by inducing expression of ICAM-1 and MHC class II on the surface of keratinocytes and by stimulating recruitment of lymphocytes to cutaneous inflammatory sites, IFN- γ may play a role in

the sensitization phase of percutaneous antigen immunization.⁹ To our knowledge, this is the first report to assess the effect of IFN- γ on modulating Th1/Th2 differentiation during sensitization phase of percutaneous protein antigen immunization.

The reason why local, but not systemic administration of IFN- γ has a modulatory effect is obscure. However, this observation is in agreement with a previous report that showed that nebulized but not parenteral IFN- γ decreased IgE production and normalized airway function in a murine model of asthma.²³ It is our speculation that locally administered IFN- γ , either by intradermal injection or patch application, could get into the epidermis and induce a change of microenvironment directly or indirectly, thus giving the out-migrating LCs enough signals to modulate Th1/Th2 differentiation when they finally arrive at regional lymph nodes to perform their antigen-presenting function. In contrast, systemic administration of IFN- γ does not access the epidermis to provide these signals in the local microenvironment. Investigations regarding the change of cutaneous cytokine patterns and surface molecule expression of LCs in regional lymph nodes are under way to assess this hypothesis. The effectiveness of epicutaneous patch-applied IFN- γ is encouraging. The horny layer of epidermis is a difficult barrier for such a hydrophilic, large protein molecule to penetrate. However, as the results suggested, the amount of IFN- γ penetrating the skin is enough to modulate the immune responses. Methods to improve percutaneous penetration of IFN- γ as well as expected modulatory effectiveness deserve further investigation.

Recombinant IFN- γ has been used to treat severe atopic dermatitis for many years. IFN- γ given by daily subcutaneous injection over a 12-week period is safe, well-accepted and effective.²⁸ Since most patients relapsed after discontinuation of IFN- γ therapy, the mechanism of effectiveness seems to be in modulation of the effector phase of immune responses. However, recently, a pilot study of IFN- γ -induced specific hyposensitization for house dust mites in atopic dermatitis was reported to be effective.²⁹ This report and our study provide highlights that IFN- γ -induced modulation of Th1/Th2 balance on the sensitization phase of percutaneous protein antigen immunization may be applied to the treatment and prevention of AD.

MATERIALS AND METHODS

Mice and reagents

Female BALB/c mice (6–10 weeks old) were purchased from the animal center of National Taiwan University, College of Medicine. All procedures performed on the mice were in accordance with The Animal Care and Use

Committee of National Taiwan University Hospital. The protein antigen used in this study was ovalbumin (OVA) (Sigma Co., St. Louis, MO, USA). OVA solution was prepared in distilled water. Recombinant murine interferon- γ was purchased from Peprotech Company (Rocky Hill, NJ, USA). The antibodies used in ELISA were all purchased from Pharmingen Company (San Diego, CA, USA).

Immunization

Mice were immunized by epicutaneous administration (patch application) of OVA.¹⁷ Briefly, for sensitization course, mice were anesthetized with pentobarbital before their backs were carefully shaven with an electric shaver. Twenty μ l of OVA solution (100 mg/ml) was first added to the paper disc insert of a Finn Chamber (Epitest LTD), which was then applied to the shaven skin, and finally fixed and secured with an elastic bandage to ensure skin contact and to prevent oral ingestion. Patches were left on for 2 days and freshly prepared patches were applied again on day 3 and day 5. All patches were removed 1 week later. In the intraperitoneal IFN- γ group, mice received 25 000 U of IFN- γ each time via the i.p. route, 2 h before patch application, on days 1, 3 and 5 of the sensitization course. In the intradermal IFN- γ group, mice received 12 500 U of IFN- γ each time via the i.d. route, 15 min before patch application, on days 1, 3 and 5 of the sensitization course. In the patch IFN- γ group, 12 500 U of IFN- γ each time was added to the paper disc insert of the Finn Chamber already containing OVA solution on days 1, 3 and 5 of the sensitization course. For challenge course which was performed 2 weeks after sensitization course, three patch administrations of OVA on alternative days were applied similarly as sensitization course, but without administration of IFN- γ in all groups of mice. For those experiments described in part III of the results, pretreatments (one or two courses) of the i.d. or patch IFN- γ with OVA were performed by similar procedures as sensitization course in two-week intervals. Repeated immunization courses were similarly performed in 2-week intervals without administration of IFN- γ in all groups of mice.

Determination of OVA-specific IgG2a and IgE levels

One week after the beginning of the challenge or repeated immunization, mice were bled and sera were prepared for determination of OVA-specific IgG_{2a} or IgE. Conventional protocols³⁰ with isotype-specific secondary antibodies (Abs) were used. An ELISA amplification system (Life Technologies, Gaithersburg, MD, USA) was included for IgE determination to improve the sensitivity. Ab titers were calculated based on linear regression analysis of absorbance. Results are expressed as ELISA titers relative to an internal isotype standard, run in each assay.

Acknowledgments

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