A NEW APPROACH FOR THE INDUCTION OF VITILIGO IN MICE

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Abstract - Tyrosinase has been recognized as a major autoantigen in human vitiligo. Here, we have shown that experimental autoimmune vitiligo can be induced by intradermal injection of mushroom tyrosinase emulsified in complete Freund's adjuvant in C57BL/6 mice. The onset of vitiligo was characterized by hair hypopigmentation and total melanocyte depletion in the basal layer of the epidermis. Our results confirm that tyrosinase is the causative autoantigen in the genesis of autoimmune vitiligo.

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Key Words: Mushroom tyrosinase, human vitiligo, experimental autoimmune vitiligo

INTRODUCTION

Vitiligo is an autoimmune disease which is found in 0.5-1% of individuals worldwide, and it is charachterized by white spots on the skin (1-3). The main target in this disease is melanocyte that could be affected by humoral and cellular immune responses (1-14). The presence of anti - melanocyte antibodies in patient's sera (1,2,4-9), in addition to CD_8^+ T cells infiltration of affected area, have also been reported (10-12). As other autoimmune diseases, genetic predisposition is proposed because of familial aggregation (2) and HLA association with disease (1,15,16). Several autoantigens on the surface of melanocyte membrane have been reported which are the target for anti - melanocyte antibodies in vitiligo including tyrosinase (1,9). Tyrosinase has been identified as the principal autoantigen on the surface of melanocytes (1,7,14) which is the key enzyme for the metabolism of melanin in pigmented cells and catecholamines in the neuroendocrine systems (1). Rapid progress can be made in the understanding of a human disorder when an experimental animal model is available. For investigating the pathogenesis of autoimmune vitiligo and it's treatment, we require a suitable animal model. The experimental models for vitiligo are the smyth chicken (17) and C57BL/ let vit.vit mice (18). Due to difficulties in accessibility to these animal models we developed, for the first time, a new approach for inducing vitiligo in mice. The vitiligo induction was achieved by using purified tyrosinase from edible mushroom (Agaricus bispora).

MATERIALS AND METHODS

Isolation and purification of mushroom tyrosinase

Tyrosinase was prepared from edible fresh white mushroom (Agaricus bispora) as described by Nelson & Mason (19). Purified tyrosinase was detected by PAGE (20) and SDS-PAGE (21) in the presence of standard mushroom tyrosinase (Sigma, USA). Enzyme activity was determined using a spectrophotometric method (22) and on electrophoretic gels as described by Gennady (23).

Immunization procedure

Six - week old female C57BL/6 mice were purchased from Pasteur Institue, Karaj, Iran. They were injected intradermally (ID) on four sites of the back skin with 50 μg tyrosinase solution prepared from mushroom and emulsified in 50 μl of Freund's complete adjuvant (Sigma, USA). Two weeks after primary immunization, the mice were injected intraperitoneally (i.p) with 50 μg mushroom tyrosinase in 50 μl of incomplete Freund's adjuvant (Sigma, USA) (24).

Histopathology

Hair hypopigmentation was observed four months after immunization. It was considered as a sign of disease. The animals were killed, and the skin areas that contained hypopigmented hairs were taken and fixed in 10% formaldehyde in phosphate buffer saline pH = 7.2, and embeded in paraffin for light microscopy. Sections were stained with hematoxylin - eosin for further investigation.

Serum antibody detection

Mice were bled from the retroorbital sinus, 7 days after i.p boosting, and the sera were tested for anti - tyrosinase specific antibodies by means of Dot ImmunoBinding Assay (DIBA).

DTH responses

Cell-mediated immunity was assessed 7 days after injection boosting by measuring the specific increment in footpad thickness 24 h after intradermal challenge with 50 μg mushroom tyrosinase in 0.05 ml, saline 0.15 M.

RESULTS

As shown in Figure 1, four months after the second immunization of female C57BL/6 mice with mushroom tyrosinase emulsified in CFA, the hypopigmented patches were found on the back hairs. In addition, 7 days after the second immunization, circulating anti - tyrosinase antibodies (Fig. 2)

Fig. 1. Development of vitiligo after two immunizations.

and in vivo cell mediated immunity (Fig. 3) to mushroom tyrosinase were produced in comparison with control mice immunized with PBS + CFA.

Histopathological study of hematoxylin and eosin - stained sections revealed a fragment of skin which showed acanthosis with total melanocyte depletion in the basal layer of the epidermis and a foreign body granuloma in the dermis composed of epithelioid histiocytes, multinucleated giant cells and mononuclear cells with dense fibro-collagenous bundles. The control group of mice immunized with PBS + CFA failed to show the histological changes described above (Fig. 4a,b).

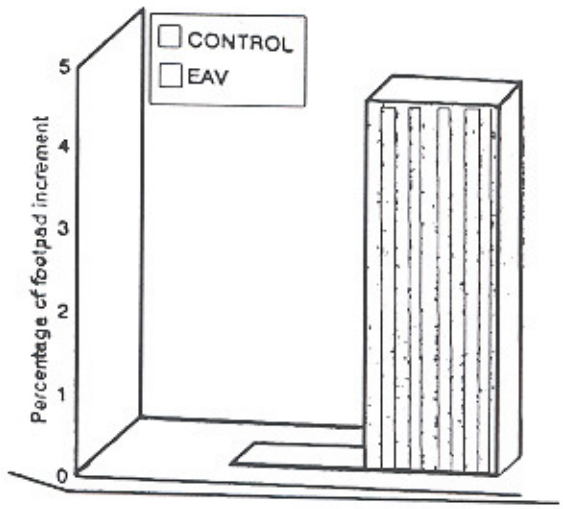

Fig. 2. Effect of immunization with mushroom tyrosinase + CFA on the production of serum anti - mushroom tyrosinase antibody. Seven days after the second immunization, mice were bled and the sera tested for anti - tyrosinase specific antibody using a Dot immunoBinding Assay. The data are presented as the geometrical mean of reversed titer (GMRT), n = 10 per group. Control animals immunized with PBS + CFA have not produced anti - tyrosinase specific antibody.

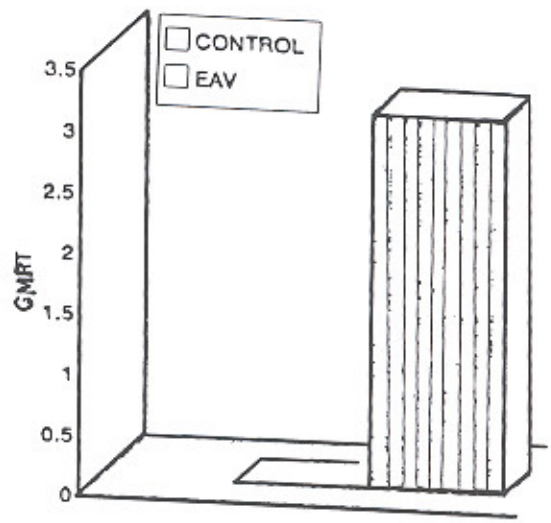

Fig. 3. Effect of immunization with mushroom tyrosinase + CFA on DTH responses. Cell-mediated immunity was assessed seven days after the second immunization by measuring the specific increment in footpad thickness 24 h after intradermal challenge with 50 μg mushroom tyrosinase, n = 10 per group. Control animal immunized with PBS + CFA did not show cell-mediated immune response.

DISCUSSION

The development of vitiligo by active direct immunization of female C57BL/6 mice with mushroom tyrosinase has not been previously reported. Our study

is the first report of autoimmune vitiligo following immunization with a purified tyrosinase autoantigen. Induction of experimental autoimmune vitiligo (EAV) by immunization with mushroom tyrosinase, suggestes that the tyrosinase could be an effective autoantigen responsible for the genesis of the vitiligo. The possible

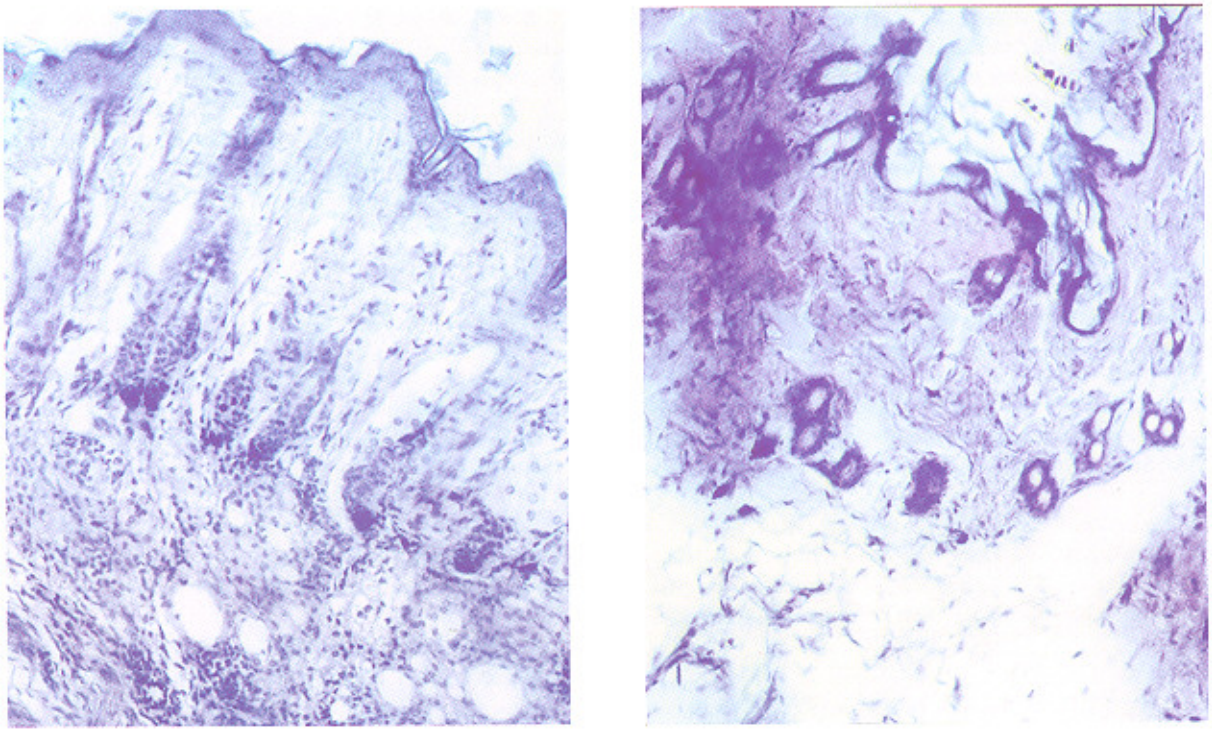

Fig. 4. Histopathology of skin of C57BL/6 mouse (a) inflammatory responses as a result of immunization with mushroom tyrosinase emulsified in CFA. The architecture of the epidermis is disorganised with the melanocyte depletion. numerous monocytic inflammatory cells are seen in the dermis (hematoxylin and eosin, original magnification X10). (b) skin of control animal.

role of tyrosinase in the etiopathogenesis of human vitiligo is further supported by the fact that patients with vitiligo show an immune response to tyrosinase (1,7). Destruction of melanocyte cells in the basal layer of the epidermis and the presence of inflammatory cellsin the dermis of EAV, indicates that the destruction of melanocytes is probably caused by mononuclear cells. In addition, the absence of overt infiltrates in the epidermis may be a consequence of dispersion of inflammatory cells throughout the basal layer of the epidermis. The clinical and histopathological appearance of vitiligo in this animal model closely resembles certain vitiligo conditions in man (12). Since, there is no satisfactory treatment for vitiligo, it seems this animal model is suitable for investigating new therapeutic approaches targeted toward various components of the immune response to control vitiligo.

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