The Serum Levels of IL-4, IL-5 and IFN-γ in Skin Allergy- and Measles- Induced Exanthema

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Abstract- Macular or maculopapular skin reactions are frequent events in skin allergies as well as in viral infections. Clinically, the differentiation may be difficult in the absence of a clear relationship with drug intake or in the failure to detect measles virus-specific antibodies. Studies on drug-specific T cell lines and T cell clones isolated from skin-allergy patients have suggested that these cells may represent a significant source of IL-4 and IL-5. On the other hand, viral infections are frequently associated with elevated IFN-γ levels. Determination of serum cytokine levels helps to differentiate between skin allergies and virally induced skin eruptions. Forty patients suffering from skin allergy and 40 patients with measles infection entered the study. Serum IL-4, IL-5 and IFN-γ levels were determined by ELISA assay for skin-allergy and measles patients. In 37/40 patients with skin allergy, IL-4 was elevated and in 6/40 patients with skin allergy IFN-γ was measurable. In 29/40 patients with measles infection, IFN-γ serum levels were elevated and 32/40 patients with measles had elevated IL-4 levels. IL-5 was increased in 32/40 patients with measles infection and in 34/40 patients with skin allergy. These data underline the distinct pathogenesis of these morphologically similar exanthemas and suggest that a combined analysis of IL-4, IL-5, and IFN-γ might help differentiate skin eruptions.

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Key words: Skin allergy, exanthema, measles, IL-4, IL-5, IFN-γ

Introduction

Macular or maculopapular skin reactions (exanthema) are frequent events in skin allergies as well as in viral infections. Their histology is similar with the exception of epidermal syncytial giant cells found in measles (1). The clinical morphology is also similar. Thus, clinically the differentiation might be difficult in the absence of a clear relationship with drug intake or in the failure to detect virus-specific antibody of IgM class. One rather characteristic criterion for skin allergy is the total-IgE amount (2, 3).

Drugs stimulate T cell either by forming hapten-protein complexes presented by MHC or possibly by direct binding to T cell receptors. It is unclear how many T cells are activated by haptens or via direct T-cell receptor (TCR) stimulation in the various forms of skin allergy (4). Activated T cells express elevated levels of CD25 and HIA-DR molecules and secret high amounts of IL-5 and IL-4 (4-6). Studies on specific T cells lines and clones of measles virus have suggested that these cells may represent a significant source of IFN-γ (7, 8). On the other hand, viral infections are frequently associated with elevated IFN-γ levels (3,9,10). IFN-γ is a critical mediator in type 1 immune response, because it stimulates macrophage activation and increases expression of major histocompatibility complex 1 and 2 molecules on antigen presenting cells (6).

In this study, we investigated whether patients suffering from skin allergy or generalized measles virus infection with exanthema have elevated IL-4, IL-5 or IFN-γ cytokine levels in their sera and whether the analysis of IL-4, IL-5 and IFN-γ might be helpful in discriminating the etiology of viral or skin allergy-induced skin eruptions (11,12). We analyzed alterations of serum cytokine levels in patients with natural measles infection

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and skin allergy.

Patients and Methods

In this prospective study 40 patients (14 females, 26 males; mean age, 36 years; age range, 16 month to 72 years) with skin symptoms due to drug or food were analyzed. Most patients consulted an out-patient clinic of dermatology. Some were hospitalized because of drug reactions and a few cases occurred because of treatments during hospitalization. Skin-allergy patients had an increase in the total-IgE. Antibody titers were kindly measured by the immunology department of Medical School, Iran University of Medical Sciences, Tehran-Iran. The sera were collected within 1 day after development of the rash.

The sera of 40 measles patients with infection (16 females and 24 males; mean age, 17.5 years; age range, 6-23 years) with an increase in the measles IgM antibody were kindly provided by National Center of Measles associated with Department of Pathobiology, School of Public Health, Tehran University of Medical Sciences, Tehran-Iran.

Sera were separated and aliquots were stored at -20º C for later measurement of cytokines. Human IL-4, IL-5 and IFN-γ were measured by a cytokine specific sandwich avidin-biotin ELISA using mouse monoclonal antibody (mAb) pair (native capture mAb and biotinylated detecting mAb, bender-med-system Vienna, Austria). In brief, flat-bottom polystyrene micro-plates was precoated with anti human cytokines were employed. Before cytokine assay, micro-plates had been washed and blocked with PBS. Serial dilutions of cytokine standards IL-4, IL-5 and IFN-γ (8-500 pg/ml for IL-4, 7.8-500 pg/ml for IL-5 and 1.5-100 pg/ml for IFN-γ) were performed with the assay buffer (PBS with 1% between 20 and 10% BSA) were produced and add to single well. Bound cytokines were detected using biotinylated antibody followed by poly-streptavidin -HRP buffer and developed with 1-step TMB coloration. Color development was measured at 450 nm. The sera were not diluted or IL-4, IL-5 and IFN-γ. Statistical analyses were performed using SPSS software (Version 11) with the significance level set at $P < 0.05$ (Mann-Whitney U tests).

Results

40 patients showed macular and maculopapular ???. IL-5 was detectable in patients with skin-allergies. IL-5 levels in all 34 patients with skin-allergy ranged between 2 and 3 pg/ml (P= 0.444; Figure 3).

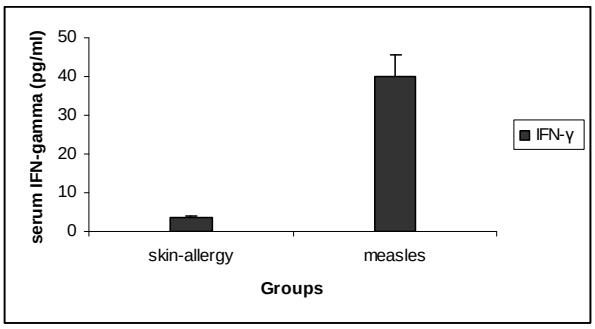

Figure 1. Mean serum IFNγ levels (Pg/ml) in patients with measles and skin allergy Mann-Whitney U test $P = 0.0001$.

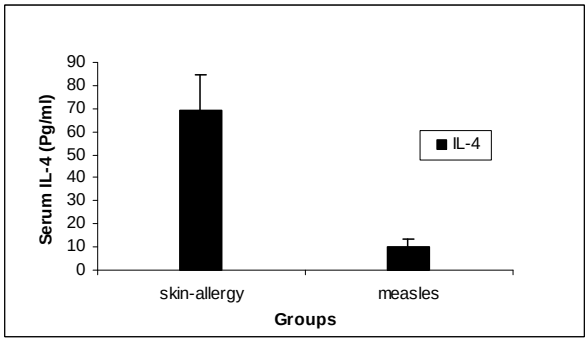

Figure 2. Mean serum IL-4 levels (Pg/ml) in patients with measles and skin allergy Mann-Whitney U test $P = 0.003$.

IL-4 serum level was detectable in the sera of 37 patients with skin-allergy. The amounts of IL-4 in 8 patients with skin-allergy ranged between 30 and 67 pg/ml, in 26 patients between 10-30 pg/ml and in 3 patients below 10 pg/ml, but still detectable (*P*, 0.0001; Fig. 2). IFN-γ level was detectable in only 6 patients with skin-allergy being lessthan 10 pg/ml. Therefore, 40 patients suffering from skin eruptions related to measles were included, who showed a macular and maculopapular (morbiliform) exanthema. The sera were collected within the 2-17 days of development of the rashes. Serum IFN-γ levels were detectable in 29 patients. These values were 50-97 pg/ml in 13 patients, 10-50 pg/ml in 12 patients and less than 10 pg/ml in 4 patients, but still detectable (*P*, 0.003; Fig. 1). Serum IL-4 levels were detectable in 32 measles patients that were below 23 pg/ml, being 10-23 pg/ml in 10 patients and below 10 pg/ml in 22 patients. IL-5 was detectable in patients with measles, the mount of which ranged 2-4 pg/ml in 32 patients.

Discussion

Our data showed that in skin-allergies, elevated IL-4 and IL-5 levels could be found and that IFN-γ was usually undetectable. On the contrary, IL-4, IL-5 and

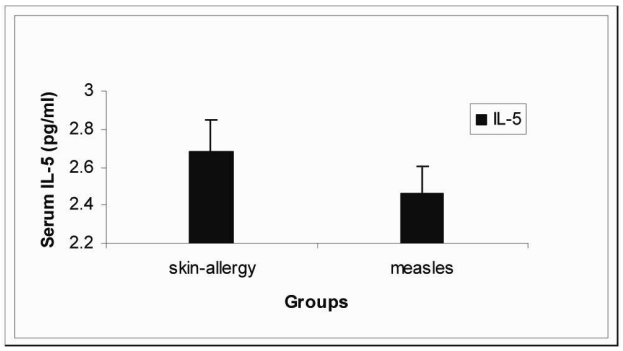

Figure 3. Mean serum IL-5 levels (Pg/ml) in patients with measles and skin allergy Mann-Whitney U test $P = 0.444$.

especially IFN-γ levels were increased in measles. Elevated IL-4 and IL-5 levels were in agreement with *in vitro* findings showing that IL-4 and IL-5 can be produced by different human peripheral T cell in skin-allergy (8, 13, 14). Determination of IFN-γ level was actually most informative in differentiating the etiology of skin rashes; it was increased in patients with measles but not in those with skin-allergies. However, skin-allergy patients did not usually have detectable IFN-γ levels, suggesting that even in these immune reactions which probably involve cytotoxic T cell (15) not all patients with skin-allergies showed elevated cytokines. It is possible that the immunostimulation was too weak to increase the cytokines. Alternatively, the cytokine levels might be only transiently increased and we might have missed optimal timing for cytokine analysis (6). In this study, we chose to compare sera of patients with measles, as this viral disease can be diagnosed definitely by the determination of anti-measles IgM and cause rashes which are clinically indistinguishable from skin-allergies. The distinct cytokine pattern suggests that in spite of the similar clinical presentation, the immunopathogenesis of skin-allergy and measles infection is different (13). IFN-γ only increased in patients with measles but IL-4 and IL-5 elevated in both groups of patients with skin-allergies and measles. The immune response during measles involved early type 1 responses, with production of IFNγ predominantly by CD^+_8T cells and CD^+_4 T cells during the rash phase of the illness**.** During and after rash phase**,** a more prolonged increase was observed in type 2 cytokines IL-4 and IL-5. Serum IFNγ levels were elevated only for 3 days after the onset of rash (1, 4**).** IFNγ is produced primarily by CD^+_8 T cells and NK cells after stimulation by measles virus. Measles virus-specific cytotoxic T cells are in circulation during the appearance of measles rashes and propose **a** role for cytotoxic T cells in virus clearance (13, 15). 3 days after the onset of rashes, another mechanism of measles virus-induced immunosuppression may result from a shift in cytokine response from a T-helper type 1 (Th1) immune response in the acute phase of measles to a predominant Th2 response during convalescence. Initial potent Th1 response accounts for viral clearance while the Th2 response supports the development of measles virus-specific antibody. In addition, IL-4, an immunoregulatory and immunosuppressive cytokine, is elevated for weeks in the serum of measles patients. IL-4 down-regulates the synthesis of cytokines, suppresses macrophage activation and T cell proliferation, and inhibits delayed–type hypersensitivity responses. IL-4 and IL-5 induce the differentiation of B cells and produce antibody (16-18). Further studies are needed to show whether the determination of cytokine levels is also helpful in discriminating other viral diseases where the clinical distinction of skin-allergy or viral etiology is difficult. In spite of clinical similarity of skin rashes in patients with viral infections and skin-allergies, they have distinct cytokine profiles in the circulation. In the absence of IFN-γ levels and the presence of IL-4 and IL-5 in skin-allergy patients and the presence of IFN-γ in patients with measles might be helpful in differentiating rashes of different etiologies. Elevated IL-4 and IL-5 in combination with low IFN-γ imply a skin-allergy induced exanthema, whereas elevated IFN-γ levels may point to a viral etiology (6, 13).

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