# Evaluation of the utility of autologous serum skin test and the efficacy of autologous serum therapy in chronic spontaneous urticaria

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*Received: 9 June 2017*
*Accepted: 26 October 2017*

**Background:** Autologous serum skin test (ASST) evaluates the presence of any serum histamine-releasing factors and histamine-releasing autoantibodies. Autologous serum therapy (AST) is a therapy in which repeated injections of autologous serum are administered intramuscularly for treatment of chronic spontaneous urticaria (CSU). The aim of this study is to evaluate the advantages and compare the results of the ASST and the efficacy of AST in CSU patients.

**Methods:** We included a total of 39 patients that presented with urticaria of more than 6 weeks duration in this study. Patients who suffered from acute urticaria, urticarial vasculitis, physical urticaria, and other systemic diseases known to cause urticaria were excluded. Standard tools and techniques were used to prepare autologous serum, injection of the serum, and interpretation of the results. The test result was implicated as positive and negative ASST.

**Results:** Out of 39 patients, 11 (27.5%) patients exhibited positive ASST reactions. Based on the urticaria total severity score (TSS), 10 patients were characterized as moderate severity whereas 29 patients were characterized as severe. There was no observed association of severity with ASST positivity. There was a significant decline in TSS at 15 weeks in both the ASST positive and negative groups. Study patients had a statistically significant response to AST, although differences between ASST positive and negative groups were not significant. This indicated the effectiveness of AST in both groups, irrespective of positive ASST results.

**Conclusion:** ASST was found to be an easy, useful, inexpensive test to detect autoimmune urticaria and classify CSU. AST is an easy, economic, and safe therapeutic tool for patients of refractory CSU with minimal discomfort, and no observed complications within the study period.

**Keywords:** autologous serum skin test (ASST), chronic spontaneous urticaria (CSU), autologous serum therapy (AST), chronic autoimmune urticaria (CAU)

Iran J Dermatol 2017; 20: 43-49

## INTRODUCTION

Urticaria is characterized by the rapid appearance of pruritic, erythematous, cutaneous elevations (wheals) that blanch with pressure. It is a common disorder of skin that affects 15%-20% of the general population [1]. Based on its duration, occurrence and causes, urticaria can be classified into three

clinical subgroups – spontaneous (80%), physical (10%), and special forms (10%).

Urticaria is a clinical reaction pattern triggered by many factors causing the liberation of vasoactive substances such as histamine, prostaglandins and kinins [2]. Clinically, urticaria is classified into acute (duration <6 weeks) and chronic (duration >6 weeks) type [3]. The pathogenesis of CSU is unclear and potential causes may include chronic infections, allergy to certain foods or food additives, anxiety, and autoantibody production against immunoglobulin E (IgE) receptor. This autoimmune subgroup, referred to as chronic autoimmune urticaria (CAU), has autoantibodies directed at the FcεR1α receptor located on mast cells and basophils, or less commonly against IgE [3].

Urticaria was previously considered to be idiopathic in most patients. Later, autoimmunity was recognized as a cause. Hide *et al.* [4] reported that autoantibodies against the high – affinity IgE receptor, FcεRIα cause histamine release in a subset of patients with chronic urticaria.. ASST is an uncomplicated *in vivo* intradermal clinical test for the detection of basophil histamine releasing activity [5]. A positive result in the form of an instant hypersensitivity reaction read against a control signifies the presence of circulating histamine releasing factors (autoantibodies) [5]. Hence, ASST is a useful diagnostic aid to identify CAU. The basophil histamine release assay is the gold standard used to detect functional autoantibodies; however, the procedure is expensive, requires fresh basophils from healthy donors, and skilled expertise. For this reason, the test is usually limited to research laboratories [6]. Identification of CAU may stimulate the use of immunotherapy in severe diseases insensitive to antihistamine therapy. In the present study, we have assessed the ASST screening test to diagnose CAU in patients with CSU of more than 6 weeks duration. In addition, we evaluated the efficacy and safety of autologous serum therapy (AST) in the management of CSU in ASST positive and negative patients.

## MATERIALS AND METHODS

This study evaluated the utility of ASST as a screening test to detect autoimmune urticaria, as a subgroup of CSU. We also assessed the efficacy and safety of AST in the management of CSU. Consecutive patients with typical signs and symptoms of CSU who attended the outpatient department of Sri Aurobindo Medical College and Post-Graduate Institute, Indore, Madhya Pradesh, India

were screened. The study was conducted over a period of 16 months, from January 2014 to April 2015. A total of 40 patients with CSU of more than 6 weeks duration who satisfied the inclusion and exclusion criteria enrolled in this study. The entire study procedure was explained to the patients who volunteered and fulfilled the selection criteria. These patients gave their written informed consent. In order to be enrolled in the study, patients should not have consumed any steroids, doxepin, or immunosuppressive drugs for at least 3 weeks and not take any other antihistaminic agents for 3 days before the ASST. The study inclusion criteria was patients with a history of wheals of more than 6 weeks duration. We excluded any patients on oral corticosteroids for the past 4 weeks, oral anti-histamines for the past 72 hours, urticarial vasculitis, history of type I hypersensitivity, physical urticaria other than simple dermographism, and pregnant and lactating women.

Venous blood was collected from the study participants in sterile plain (red top) vacutainers and allowed to clot at room temperature for 30 minutes. The serum was separated by centrifugation at 2000 rpm for 10 minutes and then aliquoted for use in the ASST. A total of 0.05 ml of the patient's own serum was injected intradermally into the left flexor forearm 2 inches below the antecubital crease and a saline control into the right forearm. Areas that had spontaneous wheals in the previous 24 hours were avoided. A reading of the wheal was taken after 30 minutes. A wheal and flare of more than 1.5 mm diameter compared with the control was considered to be positive (Figure 1). We did not administer the skin prick test with histamine diphosphate as the positive control due to risk of anaphylaxis and acute angioedema. Both ASST positive and negative patients received AST.

For the AST, 5-6 ml of blood was withdrawn and serum was separated as described above. Patients received 2 ml of serum as deep, intramuscular injections in alternating buttocks, once a week for nine consecutive weeks. For the injections, we used a disposable syringe and 22G needle. Patients

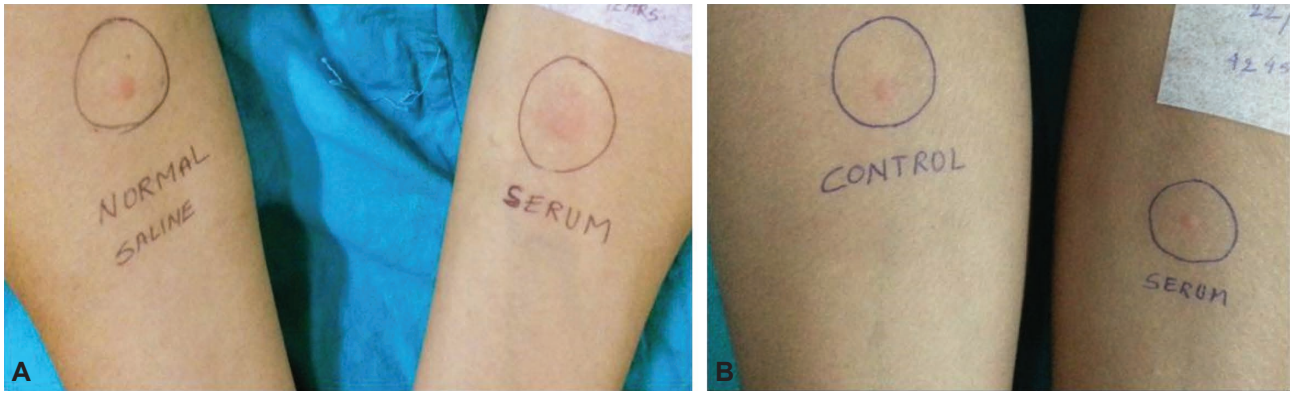


**Figure 1.** Positive (A) and negative (B) autologous serum skin test (ASST).

were evaluated by the Urticaria Total Severity Score (TSS) [7]. Patients were allowed to take an antihistamine orally (Levocetrizine, 5 mg) only if required during the study period. Evaluation of AST was performed at baseline (0 week), and weeks 9 and 15 from baseline.

We recorded 6 separate parameters for disease activity and severity according to a 0-3 scale (Table 1). Based on these, a 0-18 TSS was generated at each time point (Table 2). The enrolled patients completed the study design in 9 weeks; however, we recorded the TSS scores at the 15$^{th}$ week to assess the long-term efficacy of AST. Patients' scores were compared at weeks 0 and 15. The effect of AST was assessed on the basis of percentage of decline in urticaria TSS from the baseline score. We devised our own scale due to the paucity of established a grading system to classify and assess the response in CSU patients after receiving AST (Table 2).

### Statistical analysis

All statistical analyses were done on patients that completed all 9 autologous serum injections and were followed 6 weeks after the last injection (i.e., 15 weeks after baseline). *P*-values <0.05 were considered statistically significant.

**Table 2.** Basis of the Urticaria Total Severity Score (TSS) and autologous serum therapy (AST).

| **Assessment at baseline (0 week) according to TSS** | |
|---|---|
| TSS = 0 | Clear |
| TSS = 1-6 | Mild |
| TSS = 7-12 | Moderate |
| TSS = 13-18 | Severe |
| **Response of AST (at 9 and 15 weeks)** | |
| TSS ≥75% decline from baseline | Excellent response |
| TSS 50%-74% decline from baseline | Marked response |
| TSS 25%-49% decline from baseline | Moderate response |
| TSS <25% decline from baseline | Mild response |

## RESULTS

A total of 40 patients with CSU for more than 6 weeks were subjected to skin tests with their own serum. We observed that 11 (27.5%) patients exhibited positive ASST reactions and 29 (72.5%) patients had negative ASST reactions. However, we excluded 1 male patient with a negative ASST reaction and baseline urticaria TSS of 14. This patient had to be shifted to the other treatment modality after the 7$^{th}$ AST because he showed no response until then and also required 3 antihistamine tablets per day. The remaining 39 patients were included in the study.

Out of 39 patients, ASST was positive in 11

**Table 1.** Urticaria Total Severity Score (TSS).

| **Parameter** | **Scores** | | | |
|---|---|---|---|---|
| | **0** | **1** | **2** | **3** |
| Number of wheals | None | ≤10 | 11-50 | >50 |
| Size of wheals | None | <1 cm | 1-3 cm | >3 cm |
| Intensity of pruritus | None | Mild | Moderate | Severe |
| Duration of pruritus | None | <1 hour | 1-12 hours | >12 hours |
| Frequency of appearance | None | <Once or once per week | 2-3 times per week | Daily/almost daily |
| Frequency of antihistamine use | None | < Once or once per week | 2-3 times per week | Daily/almost daily |

(28.20%) and negative in 28 (71.79%). Patients' ages varied from 18 years to a maximum of 66 years with mean age of 30.47 years. The mean age for ASST positive patients was 35.09 years; for ASST negative patients, it was 29.14 years (Table 3). There was no association of ASST positivity to gender (*P*=0.866).

Based on the urticaria TSS (Table 2) at baseline, 3 out of 11 (27.27%) ASST positive patients had moderate disease and 8 (72.73%) had severe disease (Table 3). Among 28 patients in the ASST negative group, 7 (25%) had moderate disease and 21 (75%) had severe disease. There was no significant association of disease severity with ASST positivity.

We observed that in both the ASST positive and ASST negative groups, the mean urticaria TSS at baseline (0 week) decreased considerably at 9 weeks after completion of AST. A period of 6 weeks was allowed to elapse after completion of treatment for follow-up assessment. The same effect was maintained in both the groups until the treatment free follow-up period of 6 weeks (i.e., 15 weeks after baseline). There was a significant decline in TSS at 15 weeks. The mean baseline TSS was 13.59, whereas it was 7.74 at 15 weeks (Table 4).

The mean baseline urticaria TSS in the ASST positive group was 13.45; at 15 weeks, it was 8.09. The mean baseline TSS score in the ASST negative group was 13.64 and at 15 weeks, it was 7.61 (Table 4). There was no statistically significant difference in TSS scores at baseline and at 15 weeks between the ASST positive and ASST negative groups. This suggested that there was no difference between response in the ASST positive and ASST negative groups and that AST was effective in both groups.

**Table 4.** Autologous serum therapy (AST) in autologous serum skin test (ASST) positive and negative patients.

| | Urticaria Total Severity Score (TSS) | | |
|---|---|---|---|
| | **0 week** | **15 weeks** | ***P*-value** |
| ASST positive | 13.45±2.382 | 8.09±4.700 | 0.005 |
| ASST negative | 13.64±2.112 | 7.68±3.560 | 0.0001 |
| *P*-value | 0.808 | 0.769 | |

Based on the previously mentioned scoring at 6 weeks after AST, we noted that out of 39 patients, 5 had an excellent response (1 ASST positive, 4 ASST negative), 11 had marked response (4 ASST positive, 7 ASST negative), 14 had moderate response (2 ASST positive, 12 ASST negative), and 9 showed mild response (4 ASST positive, 5 ASST negative) as seen in Table 3. At 15 weeks (6 weeks after completion of AST), 5 patients had excellent response, 11 patients had marked improvement, 14 had moderate improvement, and 9 patients had mild improvement in CSU. None of the patients suffered any serious adverse effects. The only adverse effect was pain at the injection site, which persisted for 1-2 days. None of the patients requested analgesics.

## DISCUSSION

The aim of the present study was to evaluate the utility of ASST as a screening test to detect the autoimmune urticaria subgroup of CSU and to evaluate the efficacy and safety of AST. The intradermal injection of autologous serum in some patients with CSU produced a wheal and flare

**Table 3.** Proportion of autologous serum skin test (ASST) positive and negative cases according to sex and disease severity.

| | **ASST positive (11)** | **ASST negative (28)** | **Total (39)** | ***P*-value** |
|---|---|---|---|---|
| Age (years) | 35.09±13.63 | 29.14±9.47 | 30.82±10.95 | 0.128 |
| Male | 7 (63.64%) | 4 (36.36%) | 11 | 0.866 |
| Female | 17 (60.71%) | 11 (39.29%) | 28 | |
| Severity of disease at baseline | | | | |
| Mild | 0 | 0 | 0 | |
| Moderate | 3 | 7 | 10 | 0.8837 |
| Severe | 8 | 21 | 29 | |
| Response after autologous serum therapy (AST) | | | | |
| Excellent | 1 (20%) | 4 (80%) | 5 (100%) | |
| Marked | 4 (36.36%) | 7 (63.64%) | 11 (100%) | 0.3856 |
| Moderate | 2 (14.29%) | 12 (85.71%) | 14 (100%) | |
| Mild | 4 (44.44%) | 5 (55.56%) | 9 (100%) | |

response suggestive of histamine release factors in the serum, which later were identified as anti-FcεRI in CSU [4]. A positive ASST might indicate the presence of functional anti-IgE antibodies [8] and other histamine releasing factors could not be excluded [9,10]. At present, autoantibodies are detected by basophil histamine release assays, Western blot analysis, or ELISA, all of which are tedious methods that require specialized facilities and mostly not available to the majority of clinicians. Therefore we have decided to study the utility of ASST to identify this subgroup of CSU patients.

We enrolled 40 patients with CSU of more than 6 weeks duration for this study. A total of 39 patients completed the study. There were 11 (28.20%) patients who exhibited positive ASST reactions and 28 (71.79%) that had negative ASST reactions. This was comparable with a study by Godse [5] who reported a 26.67% incidence of autoimmune urticaria. Other studies reported 30% to 60% positive ASST [8,11]. However, a study conducted by Nimii *et al.* [9] reported that sera from approximately 60% of patients with chronic idiopathic urticaria showed a wheal that was probably attributed to histamine when injected intradermally into the patients' skin (i.e., ASST).

The ASST is a sensitive (71%) and specific (68%) test for screening CSU patients for the presence of anti-FcεRI [12]. Fagiolo *et al.* [13] supported the concept that positive cutaneous responses to autologous serum characterized a subgroup of patients who suffered from 'autoreactive' rather than 'autoimmune' chronic urticaria. Most ASST positive skin reactions occurred in chronic urticaria patients who did not express FcεRI/IgE targeting autoantibodies.

In 1999, Sabroe *et al.* [6] reported that ASST positive patients had more widespread lesions, and significantly more severe pruritus and systemic symptoms. However, the current study data confirmed observations by Nettis *et al.* [14], who found similar expressions of urticarial symptoms in ASST positive and ASST negative chronic urticaria patients.

Independent of the likely heterogeneous identity of serum factors responsible for degranulating mast cells after intradermal injections in ASST positive patients, it appeared feasible to attempt to tolerize such chronic urticaria patients to their respective circulating mast cell secretagogues [15]. The subset of chronic urticaria patients whose disease is caused by histamine releasing autoantibodies may benefit from manipulation of the immune system.

We administered AST to ASST positive and ASST negative patients. In the literature, the therapy consisted of intramuscular injections of autologous whole blood. In the current study, we have used serum instead of whole blood because of the following factors. The circulating autoreactive factor is present in the serum, rather than in the cellular components of blood. Whole blood must be injected as quickly as possible after being drawn to avoid the possibility of clotting, which requires increased patient co-operation. Finer needles can be used for injecting serum compared to those with whole blood, which reduces patient discomfort and increases compliance.

The efficacy of serum injection against whole blood injection is a possible issue. However, this seems untenable because serum is injected in the ASST and shows a positive response in the presence of the autoantibodies. This suggests that the factor that causes positive ASST is present in the serum. Hence injection of serum would also result in a similar response that is expected with a whole blood injection. We have observed that AST was well-tolerated and none of the patients reported any adverse effects except local soreness that lasted from 12 to 24 hours. We did not notice any bruising at the injection sites.

In our study, there was no difference between response in the ASST positive and ASST negative groups. Hence, AST was effective in both groups.

In a cohort study by Bajaj *et al.* [7], 35.5% of patients in the ASST positive group were completely asymptomatic at the end of the follow-up while an additional 24.2% in this group showed marked improvement. In the ASST negative group, these figures were 23 and 23%, respectively. They concluded that AST was effective in a significant proportion of ASST positive patients with chronic urticaria and a smaller number of ASST negative patients.

Chopra *et al.* [16] concluded that patients responded better to autohemotherapy compared to other modalities of treatment. Staubach *et al.* [17] performed a follow-up evaluation 4 weeks after the last (eighth) injection of autologous whole blood in their study.

Mori and Hashimoto conducted a case report in Japan of autologous whole blood (AWB) therapy

for 6 weeks in a patient with a 3-year history ofchronic urticaria. They suggested that the urticaria which existed for 3 years and cured in 6 weeks indicated the effectiveness of AWB therapy for chronic idiopathic urticaria. [15] A placebo controlled, randomized study from Istanbul reported that autologous whole blood and autologous serum injections were equally effective as compared to placebo injections in reducing disease activity in patients with CSU [18].

We do not exactly know the mechanism of AST. Therefore, we can surmise the mechanisms behind the significant improvement in approximately half of the ASST negative patients in the current study. First, is the sensitivity of ASST. Bradykinin is released when serum is separated and complement factor C5 activates to C5a. Both can cause false positive immediate type reactions. There is a poor concordance of ASST positivity with circulating antibodies to IgE or FcεRIα. Reported rates of ASST positive patients that actually have anti-FcεRIα antibodies vary from 40% [9] to <20% [17]. Hence, most patients who react to ASST do not have circulating anti-FcεRIα/IgE antibodies. On the other hand, while initial studies have reported FcεRIα positivity in ASST negative patients [9], recent studies detected these antibodies in the same number of ASST negative and ASST positive patients [17], as well as healthy controls [19] False negative results in ASST might partly explain the good response to AST seen in ASST negative CSU patients in this study.

A significant limitation of this study was its unrestrained and unblinded nature. We did not choose to use histamine as a positive control because we enrolled severely affected patients. A larger sample size and longer follow-up after AST would have allowed us to gather more data that pertained to this therapy and hence improve the results.

In conclusion, ASST was found to be an easy, useful, and inexpensive test with minimal patient discomfort. ASST reactivity might not consistently reflect the probability of response to this form of therapy. Many ASST-negative patients might benefit from this treatment. AST is an easy, inexpensive, safe therapeutic tool for patients of refractory CU with minimal discomfort and no observed complications within the study period. Response to AST in study patients was statistically significant. However, the differences between ASST positive and negative groups were not significant, which indicated the effectiveness of AST in both groups, irrespective of ASST positivity. It might also be due to the limited number of patients.

## REFERENCES


1. Sheldon JM, Mathews KP, Lovell RG. The vexing urticaria problem; present concepts of etiology and management. J Allergy. 1954; 25(6):525-60.
2. Buss YA, Garrelfs UC, Sticherling M. Chronic urticaria which clinical parameters are pathogenetically relevant? A retrospective investigation of 339 patients. J Dtsch Dermatol Ges. 2007; 5(1):22-9.
3. Grattan CE, Sabroe RA, Greaves MW. Chronic urticaria. J Am Acad Dermatol. 2002;46(5):645-60.
4. Hide M. Francis DM, Grattan CE, Hakimi J, Kochan JP, Greaves MW. Autoantibodies against the high affinity IgE receptor as a cause of histamine release in chronic urticaria. N Engl J Med. 1993; 328(22):1599-1604.
5. Godse KV. Autologous serum skin test in chronic idiopathic urticaria. Indian J Dermatol Venerol Leprol. 2004; 70(5):283-4.
6. Sabroe RA, Grattan CE, Francis DM, Barr RM, Kobza Black A, Greaves MW. The autologous serum skin test: A screening test for autoantibodies in chronic idiopathic urticaria. Br J Dermatol.1999;140(3):446-52
7. Bajaj AK, Saraswat A, Upadhyay A, Damisetty R, Dhar S. Autologous serum therapy in chronic urticaria: old wine in a new bottle. Indian J Dermatol Venereol Leprol. 2008; 74(2):109-113.
8. Grattan CEH, Francis DM, Hide M, Greaves MW. Detection circulating histamine releasing autoantibodies with functional properties of anti-IgE in chronic urticaria. Clin Exp Allergy.1992; 21(6):695-704.
9. Nimii N, Francis DM, Kermani F, O'Donnell BF, Hide M, Kobza-Black A, et al. Dermal mast cell activation by autoantibodies against the high affinity IgE receptor in chronic urticaria. J Invest Dermatol. 1996;106(5);1001-10.
10. Kermani F, Niimi N, Francis DM, O'Donnell B, Black AK, Greaves MW, *et al.* Characterization of a novel mast cell-specific histamine releasing activity in chronic idiopathic urticaria. J Invest Dermatol.1995; 105(3):452A.
11. Bakos N, Hillander M. Comparison of chronic autoimmune urticaria with chronic idiopathic urticaria. Int J Dermatol. 2003; 42(8):613-15.
12. Sabroe RA, Seed PT, Francis DM, Barr RM, Black AK, Greaves MW. Chronic idiopathic urticaria: comparison of the clinical features of patients with and without anti-FcεRI or anti-IgE autoantibodies. J Am Acad Dermatol. 1999; 40(3):443-450.
13. Fagiolo U, Kricek F, Ruf C, Peserico A, Amadori A, Cancian M. Effects of complement inactivation and IgG depletion on skin reactivity to autologous serum in chronic idiopathic urticaria. J Allergy Clin Immunol. 2000;106(3):567-72.
14. Nettis E, Dambra P, D'Oronzio L, Cavallo E, Loria MP, Fanelli M, *et al.* Reactivity to autologous serum skin test

and clinical features in chronic idiopathic urticaria. Clin Exp Dermatol. 2002;27(1):29-31

15. Mori O, Hashimoto T. Autologous whole blood intramuscular injection as a cure for chronic urticaria: report of a patient in whom intradermal injection of autologous serum continued to cause a weal-and-flare response. Br J Dermatol. 1999(6);140:1192-3
16. Chopra A, Mamta, Chopra D. Autohaemotherapy in chronic urticaria. Indian J Dermatol Venereol Leprol. 1995; 61(5):323-4.
17. Staubach P, Onnen K, Vonend A, Metz M, Siebenhaar F, Tschentscher I. Autologous whole blood injections to patients with chronic urticaria and a positive autologous serum skin test: a placebo-controlled trial. Dermatology. 2006;212(2):150–159.
18. Kocaturk E, Aktaş S, Türkoğlu Z, Kavala M, Zindanci I, Koc M, *et al.* Autologous whole blood and autologous serum injections are equally effective as placebo injections in reducing disease activity in patients with chronic spontaneous urticaria: a placebo controlled, randomized, single-blind study. J Dermatolog Treat.2012;23(6):465-71.
19. Horn MP, Gerster T, Ochensberger B, Derer T, Kricek F, Jouvin MH, *et al.* Human anti-Fc-epsilon RI alpha autoantibodies isolated from healthy donors cross-react with tetanus toxoid. Eur J Immunol. 1999; 29(4):1139-48.