

This article was downloaded by: [Uppsala University Library]

On: 20 January 2009

Access details: Access Details: [subscription number 786945401]

Publisher Informa Healthcare

Informa Ltd Registered in England and Wales Registered Number: 1072954 Registered office: Mortimer House, 37-41 Mortimer Street, London W1T 3JH, UK



Journal of Asthma

Publication details, including instructions for authors and subscription information:

<http://www.informaworld.com/smpp/title~content=t713597262>

Heat-Killed Mycobacterium bovis-Bacillus Calmette Guerin-Suppressed Total Serum IgE Response in Ovalbumin-Sensitized Newborn Mice

Mustafa Bakir ^a; Faruk Tükenmez ^a; Nerin N. Bahçeciler ^a; İl B. Barlan ^a; Müjdat M. Baaran ^a

^a Department of Pediatrics Division of Allergy & Immunology, Marmara University Hospital, Istanbul, Turkey

Online Publication Date: 01 June 2000

To cite this Article Bakir, Mustafa, Tükenmez, Faruk, Bahçeciler, Nerin N., Barlan, İl B. and Baaran, Müjdat M.(2000)'Heat-Killed Mycobacterium bovis-Bacillus Calmette Guerin-Suppressed Total Serum IgE Response in Ovalbumin-Sensitized Newborn Mice', Journal of Asthma, 37:4, 329 — 334

To link to this Article: DOI: 10.3109/02770900009055456

URL: <http://dx.doi.org/10.3109/02770900009055456>

PLEASE SCROLL DOWN FOR ARTICLE

Full terms and conditions of use: <http://www.informaworld.com/terms-and-conditions-of-access.pdf>

This article may be used for research, teaching and private study purposes. Any substantial or systematic reproduction, re-distribution, re-selling, loan or sub-licensing, systematic supply or distribution in any form to anyone is expressly forbidden.

The publisher does not give any warranty express or implied or make any representation that the contents will be complete or accurate or up to date. The accuracy of any instructions, formulae and drug doses should be independently verified with primary sources. The publisher shall not be liable for any loss, actions, claims, proceedings, demand or costs or damages whatsoever or howsoever caused arising directly or indirectly in connection with or arising out of the use of this material.

Heat-Killed *Mycobacterium bovis*-Bacillus Calmette Guerin-Suppressed Total Serum IgE Response in Ovalbumin-Sensitized Newborn Mice

Mustafa Bakir, M.D., Faruk Tükenmez, Ph.D.,
Nerin N. Bahçeciler, M.D., Işıl B. Barlan, M.D., and
Müjdat M. Başaran, M.D.

Marmara University Hospital
Department of Pediatrics
Division of Allergy & Immunology
Istanbul, Turkey

Key words: Heat-killed BCG; Mice; IgE

ABSTRACT

To determine the impact of bacillus Calmette Guerin (BCG) vaccination on IgE production in ovalbumin (OVA)-sensitized newborn mice, four groups (I, II, III, IV) of BALB/c mice were immunized on the first day of life with live BCG, killed BCG, BCG diluent, and saline, respectively. No injection was applied to mice in group V (control). All mice except group V were sensitized and challenged with OVA in the fourth and sixth weeks, respectively, and serum total IgE levels were determined at 8 weeks, 2 weeks after the second OVA challenge. IgE levels of all groups were significantly higher than the control group except for group II ($p = 0.95$). Mice in group II showed significantly lower IgE values than group IV and I ($p = 0.007$ and $p = 0.003$, respectively). We concluded that heat-killed BCG may downregulate IgE response to OVA in newborn mice.

Address correspondence to: Nerin N. Bahçeciler, Vefa Bey Sok., Yesil Apt. No. 2/B D: 39, Gayrettepe 80680, Istanbul, Turkey. Fax: +90 216 326 80 30. E-mail: aonder@ibm.net

INTRODUCTION

The prevalence of atopic diseases has been increasing in developed countries in recent decades, now affecting more than 30% of the population (1), whereas in underdeveloped countries the frequency is lower and the increase in prevalence is less (2–4). Suggested causes of this increase in prevalence include environmental changes associated with industrial development, such as increased indoor exposure to dust mites, changed indoor climate, exposure to tobacco smoke, and increased air pollution (5–8). A recently advanced hypothesis suggests that the increased prevalence of atopy in developed countries could be associated with the decline of many infectious diseases, such as tuberculosis, which induces T helper (Th)1 immune response (9). In humans Th0 cells are polarized to Th2 class in atopic and to Th1 class in nonatopic individuals in response to allergen stimulation (10,11).

The Th2 subset of lymphocytes secretes interleukin (IL) 4 and IL5 leading to enhanced production of IgE and IgG1 by B cells and eosinophilia, whereas the Th1 subset of lymphocytes secretes IL2 and interferon (IFN) gamma and helps the B cells to secrete IgG2a. In addition, IFN gamma consistently inhibits the IL4-induced IgE synthesis by B cells (12,13). If Th1 response could be augmented and/or Th2 response could be suppressed by certain factors, the outcome of immunoglobulin response would be shifted to IgG2 from IgE.

Tuberculo proteins are candidates for such a modulation; it has been shown that purified protein derivative (PPD)-specific T cell clones are in accordance with Th1 type cytokine production profile (14), and in a number of studies mycobacteria were shown to be very potent inducers of Th1 type immune response (15–17).

In many underdeveloped countries *Bacillus Calmette Guérin* (BCG), which is a live attenuated vaccine, is being administered routinely in the newborn period (18). Such an antigen stimulation may increase the proliferation of Th0 cells in favor of Th1 subset and the balance between Th1- and Th2-like response may predominantly remain in a Th1-like cytokine pattern.

Recently Shirakawa et al. demonstrated a strong inverse relationship between atopy and infection with, or exposure to mycobacterium (M) tuberculosis in Japanese children (19).

In this report we addressed the question of whether immunization of newborn BALB/c mice with live or heat-killed *Mycobacterium bovis* prior to sensitization with ovalbumin (OVA) can suppress serum IgE response.

Systemic injection of OVA adsorbed to adjuvant results in a T-cell-dependent allergen-specific IgE response and an increase of total and allergen-specific antibody titers in serum of BALB/c mice (20).

MATERIAL AND METHODS

Mice

Twelve 20-week-old pregnant BALB/c mice (provided by Scientific and Technical Research Institute of Turkey, Istanbul) were raised and maintained in a specific pathogen-free condition with an OVA-free diet. They went into labor at the 20th day of pregnancy and newborn mice were raised and maintained in the same conditions.

Immunization and Study Design

FIRST DAY OF LIFE

Newborn mice were comprised in five groups. Group I ($n = 44$) and group II ($n = 7$) mice were immunized subcutaneously on the left shoulder area with a 30-gauge needle from a tuberculin syringe with 20 μ L of live BCG and killed BCG, respectively. The same amount of BCG diluent and 0.9% saline was injected to group III ($n = 21$) and group IV ($n = 10$), respectively. Group V ($n = 17$) mice were not injected. The number of mice in group I was much higher than in the other groups because of the increased risk of death due to live mycobacteria injection.

FOURTH WEEK

All mice except group V were immunized intraperitoneally twice with 1 μ g of OVA (Sigma Chemical Co., St Louis, MO) in 4 mg

of aluminium hydroxide dissolved in 0.5 mL 0.9% NaCl, at a 2-week interval.

EIGHTH WEEK

Two weeks after the second immunization, the sensitized mice were killed by intraperitoneal injection of 0.01 mL xylazine (Rompun[®]) and 0.01 mL ketamine (Ketalar[®]), and blood samples were obtained from the right cardiac ventricle. The sera were stored at -20°C until assay.

Preparation of BCG

Lyophilized 4×10^6 CFU of *M. bovis* (BCG) Danish 1331 strain (provided by Staten Serum-institut, Denmark) was diluted with 2 mL of its own diluent containing 0.75 mg of sodium glutamate, 125 μg of magnesium sulfate, 125 μg of dipotassium phosphate, 1 mg of L-asparagine monohydrate, 12.5 μg of ferriammonium citrate, 15 mg of glycerol (85%), and 0.5 mg of citric acid monohydrate. Lyophilized 4×10^6 CFU of BCG was diluted in the same diluent and heated at 60°C for 30 min for preparation of killed BCG.

Determination of Serum Total IgE

The serum total IgE levels were determined by sandwich enzyme-linked immunosorbent assay (purified anti-mouse IgE monoclonal antibody [Pharmingen catalog no. 02111D, San Diego, CA] and biotinylated anti-mouse IgE monoclonal antibody [Pharmingen catalog no. 02122D]).

Statistical Analyses

Serum total IgE values of each group were compared by Mann-Whitney *U*-test (GraphPad Instat[™], Dr. Granger, LSU Medical Center). A *p* value of less than 0.05 was considered significant.

RESULTS

The distribution of serum total IgE values of all groups is shown in Fig. 1. The median

and range of serum total IgE levels were 148.8 (0–445) ng/mL in the sera of group I, 42.5 (2.5–60 ng/mL) in group II, 87.5 (2.5–352.5 ng/mL) in group III, 131.3 (32.5–665 ng/mL) in group IV, and 35 (0–90 ng/mL) in group V.

There was a statistically significant difference between the serum total IgE values of all groups versus control group except for group II (killed BCG), as shown in Table 1 ($p = 0.95$). Comparison of IgE values of all groups is presented on Table 2. Serum total IgE levels of mice immunized with killed BCG (group II) were found to be significantly lower than mice in groups IV (saline) and I ($p = 0.007$ and $p = 0.003$, respectively). On the other hand, no statistically significant difference was observed between the serum total IgE values of live BCG-immunized mice (group I) and mice in groups III (BCG diluent) and IV (saline) ($p = 0.06$ and $p = 0.89$, respectively). In addition, IgE values of mice in groups III and IV were not significantly different ($p = 0.186$).

DISCUSSION

To determine the impact of BCG vaccination in early life on IgE production, we immunized four groups of BALB/c mice on the first day of life with live and killed BCG, BCG diluent, and saline prior to OVA sensitization. We observed that although immunization with killed BCG suppressed the IgE response in OVA-sensitized mice, live BCG did not have the same effect. The serum total IgE values of mice immunized with killed BCG were significantly lower than those which received only saline injection or live BCG immunization.

To test whether BCG diluent itself can confer some IgE suppressive effect, group III mice were immunized with BCG diluent prior to OVA sensitization. On the other hand, mice in group IV represented the OVA-sensitized group, because the only injection applied prior to OVA sensitization was saline. The IgE levels of those two groups were not significantly different, indicating that the IgE suppressive effect of BCG was not due to the effect of the diluent.

Taken together, our results revealed that

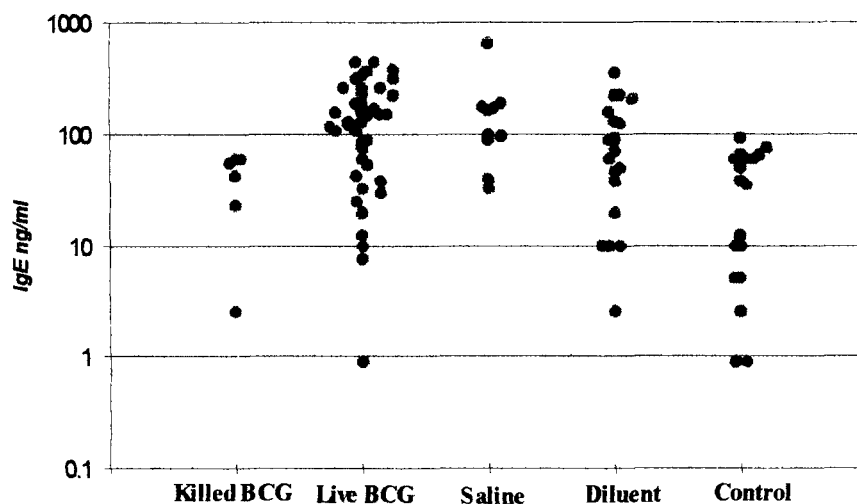


Figure 1. The distribution of serum total IgE values of all groups (logarithmic representation of the serum total IgE values).

immunization with killed BCG before OVA sensitization hindered IgE response to OVA in BALB/c mice.

Despite the increased risk of death due to injected live BCG, all animals in group I survived until the end of study. Although we had expected a better response to immunization with live BCG, we also tested the effect of killed BCG in a small group of mice. To our surprise, killed BCG demonstrated a better downregulation of serum IgE response. Our explanation of this finding is that administering live microorganisms as vaccines might have caused the number of immunized organisms to increase, and thus exceed the optimal dose for evoking Th1 response. In return, this increased dose can stimulate a mixed Th1 +

Th2 response due to increased load of antigen. Previous dose-response studies in mice identified 10^7 as the optimum dose for inducing a Th1 response to the mycobacterial antigens contained within *M. vaccae*, with no detectable Th2 component, while 10^9 evoked a mixed Th1 + Th2 response (21).

It could be speculated that, with the other factors such as geographic, environmental, racial, and antigenic variations, acquisition of frequent infections as well as administration of BCG vaccine may in part be responsible for the lower frequency of allergic diseases in underdeveloped countries in contrast to higher incidence in the developed countries.

Recently, Shirakawa et al. demonstrated a strong inverse relationship between atopy and

Table 1. Comparison of the Serum Total IgE Values of Mice in All Groups with Mice in the Control Group

MICE GROUP	NO. OF MICE	TOTAL IgE (ng/mL) MEDIAN (RANGE)	p VALUE COMPARED TO CONTROL
Live BCG (group I)	44	148.8 (0–445)	<0.0001
Killed BCG (group II)	7	42.5 (2.5–60)	0.95*
BCG diluent (group III)	21	87.5 (2.5–352.5)	0.0076
Saline (group IV)	10	131.3 (32.5–665)	0.0003
Control (group V)	17	35 (0–90)	

*Not significant (Mann-Whitney U-test).

Table 2. Comparison of the Serum Total IgE Values of All Groups

	GROUP II (KILLED BCG)	GROUP III (BCG DILUENT)	GROUP IV (SALINE)	GROUP V (CONTROL)
Group I (live BCG)	0.003*	0.063	0.89	<0.0001*
Group II (killed BCG)	–	0.055	0.007*	0.95
Group III (BCG diluent)	–	–	0.186	0.008*

*Statistically significant (Mann–Whitney *U*-test).

The numbers show *p* values of the comparison of IgE levels of groups.

infection with, or exposure to, *M. tuberculosis* in Japanese children. Positive tuberculin responses predicted a lower incidence of asthma, lower serum IgE levels, and serum cytokines shifted toward Th1 type (19). Lin et al. observed an 80% improvement in the asthmatic patients who received killed BCG by oral route (22).

In conclusion, the result of this study suggests that heat-killed BCG may downregulate the serum IgE response to OVA in newborn mice. This finding supports the hypothesis that BCG vaccination at birth may suppress Th2 response to allergens, and may have implications on the course of allergic disorders.

REFERENCES

- Weizman M, Gortmaker SL, Sobol AM, Perrin JM: Recent trends in the prevalence and severity of childhood asthma. *JAMA* 268:2673 (1992).
- Jogi R, Janson C, Björnsson E, Boman G, Björkstén B: The prevalence of asthmatic respiratory symptoms among adults in Estonian and Swedish university cities. *Allergy* 51:331 (1996).
- Von Mutius E, Weiland SK, Fritzsche C, Duhme H, Keil U: Increasing prevalence of hay fever and atopy among children in Leipzig, East Germany. *Lancet* 351:862 (1998).
- Esamai F, Anabwani GM: Prevalence of asthma, allergic rhinitis and dermatitis in primary school children in Uasin Gishu district, Kenya. *East Afr Med J* 73:474 (1996).
- Andrae S, Axelson O, Björkstén B, Fredriksson M, Kjellman N-IM: Symptoms of bronchial hyperreactivity and asthma in relation to environmental factors. *Arch Dis Child* 63:473 (1988).
- Strachan DP: Damp housing and childhood asthma: validation of reporting of symptoms. *Br Med J* 297: 1223 (1988).
- Ishizaki T, Koizumi K, Ikemori R, Ishiama Y, Kushibiki E: Studies of prevalence of Japanese cedar pollinosis among the residents in a densely cultivated area. *Ann Allergy* 58:265 (1987).
- Wickman M, Nordvall SL, Pershagen G: Risk factors in early childhood for sensitisation to airborne allergens. *Pediatr Allergy Immunol* 3:128 (1992).
- Wegman TG, Lin H, Gilbert L, Mosmann TR: Bidirectional cytokine interactions in the maternal-fetal relationship: is successful pregnancy a Th2 phenomenon? *Immunol Today* 14:353 (1993).
- Romagnani S: Regulation of the development of type-2 T helper cells in allergy. *Current Opin Immunol* 6:838 (1994).
- Romagnani S: The Th1/Th2 paradigm. *Immunol Today* 18(6):263 (1997).
- Mosmann TR, Cherwinski H, Bond MW, Giedlin MA, Coffman RL: Two types of murine helper T cell clone. I. Definition according to profiles of lymphokine activities and secreted proteins. *J Immunol* 136: 2348 (1986).
- Mosmann TR, Coffman RL: Th1 and Th2 cells: different patterns of lymphokine secretion lead to different functional properties. *Ann Rev Immunol* 7:145 (1989).
- Maggi E, Parmchi P, Manetti R, Simonelli C, Piccini MP, Rugiu FS, De Carli M, Ricci M, Romagnani S: Reciprocal regulatory effects of IFN- γ and IL-4 on the in vitro development of human Th1 and Th2 clones. *J Immunol* 148:2142 (1992).
- Mosmann TR, Sad S: The expanding universe of T-cell subsets: Th1, Th2 and more. *Immunol Today* 17: 138 (1996).
- Del Prete GF, De Carli MD, Mastromauro C, Biagiotti R, Macchia D, Falagiani P, Ricci M, Romagnani S: Purified protein derivative of mycobacteria tuberculosis and excretory-secretory antigen(s) of *Toxocara canis* expand in vitro human T cells with stable and opposite (Type 1 T helper or type 2 T helper) profile of cytokine production. *J Clin Invest* 88:346 (1991).
- Huygen K, Lozes E, Giles B, Drowaeet A, Parflet K, Jurion F, Roland I, Art M, Dufaux M, Nyabenda J: Mapping of Th1 helper T cell epitopes on major secreted mycobacterial antigen 85A in mice infected with live mycobacterium bovis BCG. *Infection Immunology* 62:363 (1994).
- Speert DP: Tuberculosis. In *Infectious Diseases of Children*, 9th ed. (Krugman S, Katz SL, Gershon AA,

- Wilfert CM, editors). Mosby Year Book, St. Louis, 1992, pp. 551–572.
19. Shirakawa T, Enomoto T, Shimazu S, Hopkin JM: The inverse association between tuberculin responses and atopic disorder. *Science* 275:77 (1997).
 20. Herz U, Lupp U, da Palma JC, Ennsle K, Takatsu K, Schnoy N, Daser A, Köttgen E, Wahn U, Renz H: The relevance of murine animal models to study the development of allergic bronchial asthma. *Immunol Cell Biol* 74:209 (1996).
 21. Hernandez P, Rook GA: The role of TNF-alpha in T-cell-mediated inflammation depends on Th1/Th2 cytokine balance. *Immunology* 82:591 (1994).
 22. Lin AY, Zhu SZ: 301 cases of bronchial asthma treated by kahusu. *Chin J Integrated Tradit West Med* 196:11(4), 209 (1991).