Int. J. Immunopharmac., Vol. 8, No. 4, pp. 433–436, 1986.
Printed in Great Britain.

0192–0561/86 $3.00+ .00
International Society for Immunopharmacology.

BRONCHO-VAXOM® AND SPONTANEOUS ALLERGIC AUTOCYTOTOXICITY (spACT) IN BRONCHIAL ASTHMA ASSOCIATED WITH FOOD HYPERSENSITIVITY

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(*Received* 19 *July* 1985 *and in final form* 11 *December* 1985)

Abstract—Spontaneous allergic autocytotoxicity (spACT) of white blood cells (WBC) was assessed in six bronchial asthma patients and eighteen normal control individuals. The observed alterations of non-primed WBC membrane were revealed as an increased uptake of trypan blue exclusion dye, an indicator of death cells. The phenomenon of spACT might be associated with a lack of T suppressor cell intervention, increased refractoriness of WBC membrane leading to its increased permeability and enhanced releasability of chemical mediators of anaphylaxis, which probably bypasses IgE events.

In six bronchial asthma patients, three were sensitive toward wheat, two had cow milk sensitivity, and one had corn sensitivity. When WBC of these patients were studied in the direct ACT assay, an additional augmentation of spACT effect by specific food antigens was observed.

Surprisingly, Broncho-Vaxom® (BX) did not inhibit or enhance spACT. However, BX has antagonistic activity toward direct ACT response in the dose-dependent concentration as previously reported.

Our preliminary clinical experience leads us to believe that the spACT assay can serve as a useful clinical discriminator of potential responders versus non-responders to therapy with new agents, when WBC disintegration by autoinduction is involved.

This study was designed to test the hypothesis that pharmacologic control mechanisms modulate spontaneous allergic autocytotoxicity (spACT) (Podleski, 1985e; Podleski, 1986). As of today, the concept that *in vivo* autoactivated cells are programmed for a suicidal reaction thus culminating in killing themselves is especially difficult to define. It is unknown whether self-inactivation represents autocytotoxic and/or autocytostatic events. Spontaneous allergic autocytotoxicity (spACT) is somewhat similar to the phenomenon called natural killing (NK) activity which is associated with natural killer lymphocytes and eosinophils (Podleski, 1985f). In this context, spontaneous cell-mediated cytotoxicity may be defined as the destruction by cells themselves in the apparent absence of sensitization or any other alteration modulated by antigen or antibody (lack of priming effect on WBC). This phenomenon has been noted by many investigators, especially in the studies of human cytotoxic systems and has been generally regarded as "background noise" serving to distract from the specificity of cell-mediated immunity. Since 1970, many excellent reviews, editorials and conferences have been published referring to this specific subject (Pross & Baines, 1977; Laux, Parker, DiSciullo, Petrarca & McAllister, 1984; Weinberg & Haney, 1983).

Therefore, it becomes apparent that in addition to direct and antibody-dependent ACT responses (Podleski, 1985b; Podleski, 1985a), a separate immune network of immediate type hypersensitivity exists, i.e. spACT, which most probably is associated with autoactivation of the cellular membrane, bypassing IgE receptor events, and culminating in cellular death.

Death in the immune response has been investigated according to the killing potential measured by effector cell and target cell interaction. It is surprising how little attention is paid to the disintegration of autologous cells in the process of anaphylactic injury. Only recently has the killing potential of mast cells and eosinophils been resurrected (Schwartz & Austen, 1984; Ayars, Altman, Gleich, Loegering & Baker, 1985; Caulfield, Lenzi, Elasas & Dessein, 1985; Podleski, 1985f).

It is well established that specific receptors for food antigens (i.e. casein) are present on human WBC (Lewis & Van Epps, 1983). At the time of oral ingestion, we are also exposed to abundant amounts of lectins. It would be reasonable to suggest that in the face of pronounced immunological reactivity, a physiological response in the form of immunosuppresssion is required to control the induction phase toward food antigens, i.e. to wheat protein antigens (O'Farrelly, Feighery, Whelan & Wier, 1984). In addition, among factors affecting the spontaneous cell-mediated cytotoxicity of intestinal mononuclear cells, impairment of gut suppressor T cells should be considered (Gibson, Hermanowicz & Jewell, 1984; Gibson, Verhaard, Selby & Jewell, 1984; Godin, Sachar, Winchester, Simon & Janowitz, 1984; Podleski, 1985c).

This study shows the immunopharmacologic modulation of Broncho-Vaxom® (BX) in spACT with comparison to direct ACT response *in vitro* in order to elucidate the potential role of bacterial ligands in the induction of tolerance toward food antigens.

EXPERIMENTAL PROCEDURES

Clinical material

Six patients with bronchial asthma (BA) were assessed for spACT and compared to eighteen normal control individuals (Podleski, 1985e; Podleski, 1986). All six BA patients have selected food hypersensitivities, three to wheat, two to cow milk, one to corn. They were also assessed with relevant food antigen in the direct ACT assay (Podleski, 1985b; Podleski, 1985d).

Separation of white blood cells

Every subject was informed of the study's purpose, and after signed consent was obtained, 5 ml of blood was collected by venipuncture into collection tubes containing EDTA as an anticoagulant. The method of Henson with minor modifications as previously described was used for separation of WBC (Podleski, 1985d; Henson, Zanolari, Schwartzman & Hong, 1978). The final differential showed 73% polymorphonuclears and 26% mononuclears.

Methodology of spontaneous allergic autocytotoxicity (spACT)

After separation of WBC, the cells of patients and controls were adjusted to a concentration of 2×10^6 cells/ml. 25 μl of WBC cell suspension and 50 μl of MH tissue culture medium (as replacement volume for food antigens and BX solutions) were mixed in one well. Using polystyrene microplates with flat bottomed microwells (Corning, No. 25860), microplates were gently shaken and incubated at 37°C for 2 h in a water bath. After incubation, to each well 25 μl of 0.1% trypan blue (TB) solution was added. The number of non-viable TB positive cells was counted directly on the invertoscope (Olympus, Model IMT).

Direct ACT microassay

As previously described (Podleski, 1985d), sterile polystyrene microplates with flat bottomed microwells (Corning No. 25860) were used for the assay. Separated WBC from patients and controls were adjusted to a concentration of 2×10^6 cells/ml. Cow milk, wheat and corn lyophilized antigens were resuspended in 1 ml of MH and further dilution was made in a concentration of 10^{-1} w/v. 25 μl WBC cell suspension, 25 μl of food antigen solution, and 25 μl of MH (as replacement volume for BX preincubation) were mixed in one well in triplicate. Proper controls were established. Microplates were gently shaken and incubated at 37 °C for 2 h in a water bath. After incubation to each well, 25 μl of 0.1% TB solution was added. The number of non-viable TB-stained cells was counted directly on the invertoscope (Olympus, Model IMT).

Modulation of spACT and direct ACT by BX; microassay

Patient and control WBC were preincubated with 25 μl of BX in concentrations of 1×10^{-10} to 1×10^{-7} mg/ml for 15 min at 37 °C. Broncho-Vaxom® (BX) was provided by OM Laboratories Ltd., Meyrin-Geneva, Switzerland in the form of a standardized lyophilizate which contains 17.5% of active principle consisting of bacterial lysate obtained from *Haemophilus influenzae, Diplococcus pneumoniae, Klebsiella pneumonia et ozaenae, Staphylococcus aureus, Streptococcus pyogenes et viridans, Neisseria catarrhalis.*

Subsequent studies of spACT and direct ACT assay were conducted according to the above method with the exception that 25 μl of MH was not added for the incubation period in order to keep the same volume of the incubation mixture. The number of TB positive, non-viable cells was counted after incubation time in order to assess the inhibitory capacity of BX in the ACT assays.

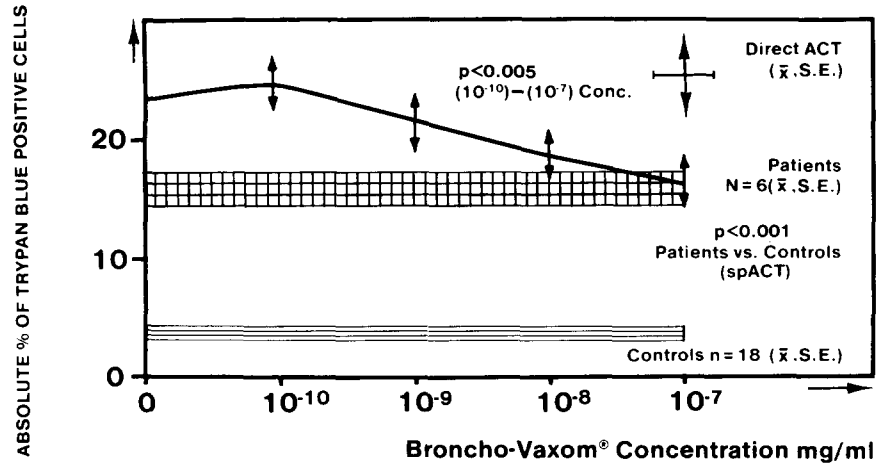

Fig. 1. Effect of Broncho-Vaxom^R on direct and spontaneous allergic autocytotoxicity. Horizontal lines (bottom) represent spACT values among eighteen control individuals (mean, S.E.). Hatched lines represent values of spACT among six BA patients (mean, S.E.). Continuous lines (top) represent direct ACT values among six BA patients inhibited with different concentrations of BX. For statistical analysis, see Experimental Procedures and Results.

Calculation of results for spACT and direct ACT

The absolute percentage of non-viable trypan blue positive cells was expressed by counting at least 200 cells/well. In each experiment, the mean of the triplicate was calculated. Every patient and every control had at least three separate experiments on three separate occasions.

Statistical analysis

Percent of inhibition data were assessed using the *t*-test, paired and unpaired. The results have been expressed as means and standard error (S.E.).

RESULTS

Analysis of our data clearly shows that spACT revealed by increased permeability of cellular membrane to trypan blue is not inhibited or enhanced with BX.

Additional experiments were performed when the patients were assessed in direct ACT assay (Podleski, 1985b; Podleski, 1985d), when relevant food antigen was introduced to the culture medium. It became apparent that in addition to the high spACT, the patient reveal also direct ACT. The difference in absolute percentage of TB positive cells between spACT and direct ACT was statistically significant ($P<0.001$). Therefore, it can be reasonably postulated that spontaneous and direct ACT are the two distinctive phenomena, mediated by different mechanisms.

Figure 1 shows the inhibitory capacity of BX in the assessment of spontaneous and direct ACT. It has been shown previously in our institution that BX exerts a very potent inhibitory capacity on direct and antibody-dependent ACT in the concentration of 10^{-10} to 10^{-7} mg/ml (Podleski, 1985d). As shown in Fig. 1, these results are confirmed ($P<0.005$). The observed direct ACT is inhibited by BX in the dose responding concentration. It is very clear, however, that spACT is not affected by BX.

DISCUSSION

The role of bacterial ligands in the induction of oral tolerance toward food antigens is of paramount importance for sensitive hosts. Since the food and bacterial flora in the gut present a major antigenic challenge to allergic individuals, the interactions of these antigens were investigated in the direct and antibody-dependent ACT assay (Podleski, 1985d). It has been observed that Broncho-Vaxom^R (BX), a potent immunostimulator of gut-associated lymphoid tissue (GALT), inhibits direct and antibody-dependent ACT (Maestroni & Losa, 1984; Podleski, 1985d). Thus, altered food antigen response absorbed from the intestine might be

corrected by BX, presumably by induction of suppressing mechanisms in GALT (Podleski, 1985g).

In the present study, it becomes evident that BX does not inhibit or potentiate spACT. This observation might assist in the explanation of why BX inhibits only direct ACT and not spACT. If spACT is mediated by a new, unknown pathway of anaphylactic injury, not associated with immunopharmacological profiles of BX, then its therapeutic role among spACT positive patients is dubious. The population of spACT positive patients may represent a distinctive subpopulation of BA patients with failure to BX treatment. Therefore, spACT assay could represent a very valuable discriminatory *in vitro* tool available to select potential clinical responders vs non-responders toward BX therapy. Additional clinical study *in vivo* will elucidate this phenomenon.

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