studies by Free and Spencer-Booth [13] support our observations of within-colony hsp induction: survivorship of bees exposed to 45–47 °C is higher among bees previously acclimated to normal colony temperatures (35 °C) than bees initially held at ambient temperature (20 °C).

We hypothesize that slight hsp induction within the normal colony may provide an adaptive mechanism for resisting the potential adverse effects of colony overheating. Although the functional significance of the heat-shock response is presently uncertain, the response may be a ubiquitous mechanism for maintaining cellular homeostasis. There is clear evidence at both the cellular and organismal levels that induction of hsp synthesis is frequently correlated with the acquisition of thermotolerance. That is, an initial exposure to mild heat-shock conditions confers increased survivorship to previously lethal temperatures [2, 3, 14, 15]. Within-colony hsp induction (and subsequent acquisition of thermotolerance) may be important to honey bees not only during warm weather, but also in cold climates as colony survival is dependent upon heat production by individual bees (primarily by microvibration of flight muscles) within the cluster [16]. Thoracic temperatures of these bees at times undoubtedly exceed 40 °C in order to maintain cluster temperatures of 33–34 °C. Core temperatures of 40.5 °C have been reported under cold weather conditions [5].

There is also a growing body of evidence that hsps can be induced by a variety of other stress treatments [15]. Agents that do induce hsp synthesis also frequently convey thermotolerance to the organism [1–3]. Most importantly, cross-resistance among various inducing agents is often observed: heat-shock, for example, induces tolerance to ethanol, anoxia and several other forms of stress [2]. If hsp induction in the honey bee proves to be associated with various environmental stimuli, observations of levels of induction may provide a vehicle for monitoring the effects of various stress-inducing agents on colony dynamics.

1 Schlesinger, M. J., Ashburner, M., and Tissieres, A., (eds), Heat Shock from Bacteria to Man. Cold Spring Harbor, New York 1982.
2 Lindquist, S., A. Rev. Biochem. *55* (1986) 1151.
3 Lindquist, S., and Craig, E. A., A. Rev. Genet. *22* (1988) 631.
4 Hunt, C., and Morimoto, R. I., Proc. natl Acad. Sci. USA *82* (1985) 6455.
5 Owens, C. D., USDA-ARS Tech. Bull. 1429 (1971) 1.
6 Southwick, E. E., J. comp. Physiol. *156B* (1985) 143.
7 Southwick, E. E., Comp. Biochem. Physiol. *75A* (1983) 641.
8 Ashburner, M., and Bonner, J. J., Cell *17* (1979) 241.
9 DiDomenico, B. J., Bugaisky, G. E., and Lindquist, S., Proc. natl Acad. Sci. USA *79* (1982) 6181.
10 Severson, D. W., Williamson, J. L., and Aiken, J. M., Insect Biochem. *19* (1989) 215.
11 Haydak, M. H., A. Rev. Ent. *15* (1970) 143.
12 Fleming, J. E., Quattrocki, E., Latter, G., Miquel, J., Marcuson, R., Zuckerkandl, E., and Bensch, K. G., Science *231* (1986) 1157.
13 Free, J. B., and Spencer-Booth, Y., Ent. exp. appl. *5* (1962) 249.
14 Neidhardt, F. C., VanBogelen, R. A., and Vaughn, V., A. Rev. Genet. *18* (1984) 295.
15 Nover, L., (ed.), Heat Shock Response of Eukaryotic Cells. Springer-Verlag, New York 1984.
16 Esch, H., Z. vergl. Physiol. *43* (1960) 305.

0014-4754/90/070737-03$1.50 + 0.20/0

Production of active and passive anaphylactic shock in the WBB6F$_1$ mouse, a mast cell-deficient strain

A. Arimura, M. Nagata, A. Watanabe, K. Nakamura, M. Takeuchi and M. Harada*

Shionogi Research Laboratories, Shionogi & Co., Ltd., Fukushima-ku, Osaka 553 (Japan)
Received 20 November 1989; accepted 9 January 1990

Summary. The role of mast cells in active and passive anaphylactic shock was examined using the WBB6F$_1$ mouse, a genetically mast cell-deficient strain. Lethal anaphylactic shock occurred at high incidence rates in mice actively sensitized to bovine serum albumin (BSA). The reaction was specific to BSA since the shock could not be elicited by human or guinea pig serum albumin in these animals. Lethal shock could be prevented by CV-3988 but not by cyproheptadine, which suggests that the shock is mediated by PAF but not by histamine and serotonin. Similarly, lethal shock was provoked by homologous antigens in mice which had been passively sensitized with allogeneic anti-benzylpenicilloyl (BPO) IgG$_1$ monoclonal antibody or with allogeneic or xenogeneic anti-BSA antiserum, but not in those sensitized with allogeneic anti-BPO IgE monoclonal antibody. These findings suggest that mast cells are not necessarily required for anaphylactic shock in the mouse.
Key words. WBB6F$_1$ mouse; anaphylactic shock; IgE antibody; IgG$_1$ antibody; xenogeneic antibody; mast cell-deficiency.

Type I hypersensitivity reactions, such as anaphylactic shock and passive cutaneous anaphylaxis (PCA), is caused by histamine, serotonin or other chemical mediators released by antigen-antibody interaction from mast cells and basophils [1]. The role of mast cells would be particularly important in the mouse since, in general, few basophils are present in mouse blood [2]. However, it has recently been found that anaphylactic shock in mice is

not mediated by histamine and serotonin but by PAF [3,4]. This comes from the finding that lethal anaphylactic shock was markedly suppressed by a PAF antagonist, CV-3988, but not by cyproheptadine, a potent antagonist to histamine and serotonin. In view of the fact that PAF can be released from a variety of cell types such as polymorphonuclear leukocytes (PMN), macrophages and epithelial cells rather than from mast cells [5], mast cells may not always be required for anaphylactic shock in the mouse. The present study was conducted to examine the role of mast cells in mouse anaphylactic shock using the $WBB6F_1$ mouse, a strain genetically deficient in mast cells [6].

Materials and methods

1. Animals. Female $WBB6F_1$ mice aged 7–8 weeks were purchased from Shizuoka Laboratory Animal Center, Hamamatsu, Japan. They were used as test animals for active and passive anaphylactic shock. WS rats (inbred, female, bred at Shionogi Aburahi Laboratories, Shiga, Japan, Shi), NOD mice (female, 15 weeks of age, Shi) and a rabbit (female, 2.5 kg, Kitayama Labes, Kyoto, Japan) were employed as the producers of antibody to bovine serum albumin (BSA). DS mice (inbred, female, 8 weeks of age, Shi) were used for PCA tests to determine IgE and/or IgG antibody titers of mouse ascitic fluids or antisera of mouse, rat and rabbit origin.

2. Antigens and antibodies. Mouse ascitic fluid containing monoclonal IgE or IgG_1 antibody to benzylpenicilloyl (BPO) haptens prepared in our laboratory, i.e., BIE-13CE or BIG-3N, respectively [7], was used. As an elicitor homologous to these antibody preparations, a multivalent conjugate of BPO haptens with guinea pig serum albumin, BPO_{17}-GpSA, was employed. Preparation of the conjugate was done according to Levine et al. [8], and determination of its hapten content was according to Ebata et al. [9]. Details have been described elsewhere [10]. Also, BSA (Nakarai Chemicals, Kyoto, Japan) and anti-BSA antisera raised in NOD mice and WS rats, and a rabbit (preparation, see below) were used. To test the antigen specificity of shock, human serum albumin (HSA, Sigma Chemical Co., St. Louis, MO, USA) and guinea pig serum albumin (GpSA, Sigma Chemical Co.) were employed.

3. Preparation of anti-BSA antisera. NOD mice, WS rats and a rabbit were immunized by i.p. (mice and rats) or i.m. (rabbit) injection of 1 mg (mice and rats) or 5 mg (rabbit) of BSA emulsified with Freund's complete adjuvant (FCA, Difco, Detroit, Mich., USA). Injections were given twice, with an interval of 2 weeks between them, and the animals were bled 2 weeks after the 2nd injection. Antisera of 15 NOD mice and 5 rats were pooled separately.

4. Determination of antibody titers of antisera and ascitic fluids. IgE and IgG_1 antibodies of mouse antiserum and ascitic fluids were titrated by menas of 1-day and 1-h PCA in DS mice, respectively. IgG antibodies of rat and rabbit origin were also titrated by 1-h PCA in DS mice. The recipients were injected i.v. with 1 mg of the homologous elicitor (0.2 ml) with 0.5 mg of Evans blue. The maximal dilution of each antibody preparation giving a blue patch with a diameter more than 5 mm was taken as its antibody titer.

5. Active anaphylactic shock. $WBB6F_1$ mice were immunized by a single i.p. or s.c. injection of 1 mg of BSA (0.2 ml) either with FCA or killed *Bordetella pertussis* organisms (Bp, 2×10^9 cells, Nakarai Chemicals), and 1–2 weeks later shock was provoked by injecting 1 mg of each elicitor. The animals were observed for 2 hours, although lethal shock was usually provoked within 30 min after the antigen challenge.

6. Passive anaphylactic shock. $WBB6F_1$ mice were injected i.v. with 0.2 ml of the BIE-13CE (anti-BPO IgE antibody) or BIG-3N (anti-BPO IgG_1 antibody) ascitic fluids. The PCA titers of these two ascitic fluids were comparable to each other in DS mice, the titer of BIE-13CE being 1:1024–1:2048 and that of BIG-3N 1:2048. The mice thus sensitized for 1 day (BIE-13CE) or 3 hours (BIG-3N) were injected i.v. with BPO-GpSA (1 mg, 0.2 ml) to provoke anaphylactic shock. Similarly, the mice sensitized for 3 h with two-fold serial dilutions (0.2 ml) of the anti-BSA antiserum of NOD mouse, rat or rabbit were tested for anaphylactic shock by challenge with BSA (1 mg). PCA titers of the anti-BSA sera of NOD mouse, WS rat and rabbit origin were 1:4096, 1:512 and 1:512, respectively. In some experiments, the mice were treated with an intraperitoneal injection of Bp (2×10^9 cells) to enhance the susceptibility to shock 1 week before it was provoked.

7. Drug treatments. To test the suppressive effects of antagonists to possible chemical mediators, the mice actively sensitized to BSA with FCA or Bp were given cyproheptadine (5 mg/kg, i.p., Sigma Chemical Co.) or CV-3988 (3–30 mg/kg, i.v., supplied by Dr S. Kamata of Shionogi Research Laboratories) at 30 or 5 min before the antigen challenge, respectively.

Results

1. Production and inhibition of active anaphylactic shock. As shown in table 1, a number of $WBB6F_1$ mice immunized against BSA using FCA or Bp as an adjuvant died of shock when i.v. challenged with BSA. Death occurred 10–30 min after the antigen challenge. The animals immunized against BSA did not show any shock symptoms

Table 1. Production of active anaphylactic shock in $WBB6F_1$ mice

Immunogen injection	Time of test	Lethality by BSA	HSA	GpSA
BSA + FCA i.p.	Day 7	1/5		
	Day 14	13/20		
BSA + FCA s.c.	Day 10	5/5	0/5	0/5
BSA + Bp i.p.	Day 7	3/5		
	Day 10	5/10		
	Day 14	13/15	0/5	0/5

Table 2. Suppression of active anaphylactic shock

Drug	Dosage (mg/kg)	Route	Time of injection[a]	Lethality[b] BSA + FCA	BSA + Bp
Control (saline)		i.v.	5 min	8/14	9/10
CV-3988	30	i.v.	5 min	0/5	NT[c]
	10	i.v.	5 min	0/5	1/5
	3	i.v.	5 min	0/5	0/5
Cyproheptadine	5	i.p.	30 min	5/10	8/10

[a] Before the antigen challenge, [b] Tested 2 weeks after the immunization (i.p.), [c] NT: Not tested.

Table 3. Production of passive anaphylactic shock in $WBB6F_1$ mice

Antibody preparation	Dilution	Lethality[a] − BP	+ Bp
Anti-BPO IgG_1 ascites (BIG-3N)	1:1	7/10	3/3
Anti-BPO IgE ascites (BIE-13CE)	1:1	0/4	0/5
Anti-BSA NOD mouse serum	1:1	2/4	
	1:2	4/4	
	1:4	4/4	2/2
	1:8	2/3	
Anti-BSA rat serum	1:1	4/4	
	1:2	3/4	
Anti-BSA rabbit serum	1:1	2/3	
	1:2	1/3	

[a] Shock was elicited by the homologous antigen.

when given HSA or GpSA as an elicitor, which indicates that the lethal shock observed was specific to BSA. Lethal shock was markedly suppressed by CV-3988 but not at all by cyproheptadine (table 2).

2. Passive anaphylactic shock. Lethal anaphylactic shock was also provoked by BPO-GpSA in most $WBB6F_1$ mice which had been sensitized passively with allogeneic anti-BPO IgG_1 monoclonal antibody, regardless of whether or not Bp treatment had been given (table 3). Generally, death occurred within 30 min after the antigen injection. Because GpSA alone provoked no shock symptoms, it is evident that lethal shock was caused by the interaction between BPO haptens and anti-BPO antibodies. Similar results were obtained with passive anaphylactic shock mediated by allogeneic and xenogeneic anti-BSA antisera. In the case of xenogeneic antisera, lethal shock was elicited at high incidence, but death was somewhat delayed; most mice were dead 30–60 min after the antigen challenge. Passive shock was also specific, in general, to the antigen homologous to the antibody used. Only in mice sensitized with anti-BSA antiserum of NOD mouse origin was lethal shock evoked not only by BSA but also by GpSA (data not shown). In contrast, lethal shock was not elicited by BPO-GpSA when mice had been sensitized with anti-BPO IgE monoclonal antibody, irrespective of pretreatment with Bp.

Discussion

Our results show that lethal shock can be produced at high incidence by the homologous antigen in $WBB6F_1$ mice sensitized by active or passive means. $WBB6F_1$ mice are known to be deficient in mast cells, owing to a lack of the appropriate precursor cells[6]. We confirmed this with the mice we used, finding no mast cells by toluidine blue staining of the skin and of peritoneal exudate cells (data not shown). Also, histamine, a biochemical marker of mast cells, was not detected in either the supernatant or the pellets of peritoneal exudate cell suspensions (data not shown). The lethal shock observed was a real anaphylactic reaction and not due to the toxic effect of eliciting antigens, because the shock was not elicited when the elicitor was not homologous to the antibody used. Lethal shock by heterologous antigen could only be elicited when mice sensitized with anti-BSA antiserum of NOD mouse origin were injected with GpSA. This was attributed to the cross-reactivity of the antibody, since this antiserum produced PCA in DS mice in collaboration with GpSA (PCA titer 1:256) as well as with BSA (PCA titer: 1:4096). In contrast, anti-BPO IgE antibody did not sensitize $WBB6F_1$ mice for anaphylactic shock. This was the case with both Bp-pretreated and -untreated animals. Because both ascitic fluids, BIE-13CE and BIG-3N, displayed similar PCA titers in DS mice, it seems unlikely that the amount of the IgE antibody was insufficient.

These results indicate that mast cells are not necessarily required for anaphylactic shock in the mouse. Although it is generally accepted that the principal target cells for both allogeneic IgE and IgG_1 antibodies are mast cells[11], the present findings show that the IgG_1 antibody is able to sensitize other cells as well. Basophils may seem a possible target but are most likely not, because we confirmed that only a few basophils (10–20 cells/μl) are present in the blood of $WBB6F_1$ mice as well documented in other strains[2]. Thus, what cell types are responsible for the anaphylactic shock in $WBB6F_1$ mice is not clear at this time. However, in view of the finding that CV-3988, a specific antagonist to PAF, suppressed anaphylactic shock (table 2 and references 3 and 4), some cells such as PMNs, macrophages and endothelial cells of blood vessels may be involved, because these cells are known as the main source of PAF[5]. The answer awaits further work.

* Author for correspondence.

1 Ishizaka, T., and Ishizaka, K., Prog. Allergy *19* (1975) 60.
2 Galli, S. J., Dvorak, A. M., and Dvorak, H. F., Prog. Allergy *34* (1984) 1.
3 Terashita, Z., Imura, Y., Shino, A., and Nishikawa, K., J. Pharmac. exp. Ther. *243* (1987) 378.

4 Arimura, A., and Harada, M., Lipids, in press.
5 Barnes, P. J., Fan Chung, K., and Page, C. P., J. Allergy clin. Immun. *81* (1988) 919.
6 Kitamura, Y., Go, S., and Hatanaka, K., Blood *52* (1978) 447.
7 Fukushima, H., Misaki, R., Takeuchi, M., Niinomi, Y., and Harada, M., Clin. exp. Immun. *68* (1987) 427.
8 Levine, B. B., and Ovary, Z., J. exp. Med. *114* (1961) 875.
9 Ebata, M., Miyake, Y., and Uchida, J., J. Antibiot. *29* (1976) 665.
10 Harada, M., Takeuchi, M., and Watanabe, A., Chemotherapy *35* (S-1) (1987) 435.
11 Vaz, N. M., and Prouvost-Danon, A., Prog. Allergy *13* (1969) 111.

0014-4754/90/070739-04$1.50 + 0.20/0

γ-Glutamyl-transpeptidase in lymphatic tissues of *Mastomys natalensis* during an infection with *Acanthocheilonema viteae* *

S. N. Singh[a], A. K. Srivastava, S. C. Gupta, R. K. Chatterjee[a] and K. C. Saxena

Divisions of Biochemistry and [a]Parasitology, Central Drug Research Institute, Lucknow 226001 (India)
Received 3 October 1989; accepted 9 January 1990

Summary. During *Acanthocheilonema viteae* infection, the specific activity of γ-glutamyltranspeptidase (γ-GT) increased in peritoneal exudate cells and bone marrow and decreased in lymphnodes of *Mastomys natalensis* throughout the course of infection. However, though there was an increase in specific activity of γ-GT in thymus and spleen during the prepatent phase of *A. viteae* infection, the level either returned to normal or decreased during the latent phase of infection. A close correlation was observed between the host's immune status during *A. viteae* infection and the level of γ-GT in lymphoid tissues.
Key words. Acanthocheilonema viteae; Mastomys natalensis; γ-glutamyl-transpeptidase; lymphoid tissues; filarial infection; immunosuppression.

The enzyme γ-glutamyl transpeptidase (EC 2.3.2.2.; γ-GT), which catalyses the initial step in the breakdown of glutathione, i.e. the transfer of the γ-glutamyl moiety of glutathione to a variety of amino acids and other peptides, has attracted attention because of its presence in lymphoid cells[1,2]. The activity of γ-GT was found to be 2–3 times higher in B-lymphocytes than in T-lymphocytes, and an enhancement of the activity of γ-GT was also reported after mitogenic stimulation of lymphocytes[1]. Marked variations in the γ-GT activity in lymphoid cells derived from patients with various neoplastic and lymphoproliferative diseases have also been reported[2]. So far, there has been no report on γ-GT activity in the host's lymphoid tissues during parasitic infections. *Acanthocheilonema viteae* infection in *Mastomys natalensis* is a chronic filarial infection, and resembles human onchocerciasis. We examined the specific activity profile of γ-GT in certain lymphoid and non-lymphoid tissues of *M. natalensis* at various stages of *A. viteae* infection. The results obtained are summarised in the present communication.

Materials and methods

Mastomys natalensis 'GRA', Giessen strain, were infected with *Acanthocheilonema viteae* through the infective bites of the tick vector *Ornithodoros moubata*. Details of maintenance and monitoring of the infection were as described elsewhere[3]. At all stages of infection non-infected animals of matched age were used as controls.

For tissue collection, the animals were killed by cervical dislocation. Lymph nodes (popliteal and mesenteric), spleen, thymus and liver of groups of animals which had prepatent, patent and latent *A. viteae* infections, as well as control animals, were excised, washed in cold 150 mM KCl and homogenized in the same medium (10 %, w/v). Exudate cells from the peritoneal cavity, and bone marrow from femur and tibia bones, were collected in phosphate buffered saline (pH 7.2). Peritoneal exudate cells (PEC) and bone marrow cells (BM) were recovered by centrifugation at low speed (500 × g 10 min) and suspended in 2.0 ml of 150 mM KCl. The homogenates of thymus, spleen, lymph nodes and liver, as well as PEC and BM suspensions, were sonicated at 1.5 Å output in a Cell Disruptor Model W-220 F (Heat Systems – Ultrasonics, USA) for 1 min and centrifuged at 10,000 × g for 30 min, and the supernatants were assayed for γ-GT activity.

The specific activity of γ-glutamyl transpeptidase was determined by previously described methods using the artificial substrate γ-L-glutamyl-p-nitroanilide[4]. 1 ml of assay mixture contained 100 mM Tris (pH 8.0), 20 mM glycylglycine, 5 mM γ-glutamyl-p-nitroanilide and a suitable amount of the enzyme preparation. The reaction vessels were incubated at 37 °C for 30 min. After inhibition of the reaction with 1.5 N acetic acid, the formation of p-nitroaniline was monitored at 405 nm and the enzyme activity expressed as μg p-nitro-aniline liberated $min^{-1}mg^{-1}$ protein.