Ya, the gene controlling expression of the H-Y antigen as defined by transplantation and cytotoxic T cell assays (*10*) and a closely linked spermatogenesis gene (*24*). It has been suggested that the genes controlling H-Y expression and spermatogenesis are the same (*24*). It is tempting to speculate that the expressed *Zfy-2* gene is *Hya* or the spermatogenesis gene. The exact functions of *Zfy-1* and *Zfy-2*, however, cannot be elucidated without further molecular characterization and functional assessments, such as transgenic mouse construction or gene inactivation by targeted gene transfer.

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Pavlovian Conditioning of Rat Mucosal Mast Cells to Secrete Rat Mast Cell Protease II

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Antigen (egg albumin) injections, which stimulate mucosal mast cells to secrete mediators, were paired with an audiovisual cue. After reexposure to the audiovisual cue, a mediator (rat mast cell protease II) was measured with a sensitive and specific assay. Animals reexposed to only the audiovisual cue released a quantity of protease not significantly different from animals reexposed to both the cue and the antigen; these groups released significantly more protease than animals that had received the cue and antigen in a noncontingent manner. The results support a role for the central nervous system as a functional effector of mast cell function in the allergic state.

SEVERAL STUDIES SUGGEST THAT ANAphylaxis can occur in the absence of any antigenic stimulus or physical irritant (*1, 2*) and may represent a conditional response (*2*). That is, neutral stimuli, such as sensory events (conditional stimulus; CS) may become associated with antigens such that subsequent exposure to these stimuli will elicit anaphylactic responses. In Pavlovian conditioning terms, the antigen is an unconditional stimulus (US) that elicits an unconditional response, anaphylaxis.

That the immune system is influenced by Pavlovian conditioning (*3*) has provided evidence for nervous-immune system interactions. Cues paired with both immunosuppressive (*3*) and immunoenhancing agents (*4*) induce conditional immunomodulation. Conditional alterations of immune function also occur in animals trained with antigens as the US (*5*). Conditional secretion of histamine in the guinea pig was previously reported, although the cellular source of the conditional histamine release was not identified (*5*). This suggested that mast cells, which are targeted in allergic disease (*6*), might be influenced by the nervous system. Evidence to support this hypothesis was lacking, however, as histamine may be released from a variety of cells at many sites within the body (*5*). We measured a protease specific to mucosal mast cell activity and demonstrated that mucosal mast cell activity is subject to central nervous system modulation.

Mast cells have high-affinity binding re-

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ceptors for immunoglobulin E (IgE), and mediate anaphylactic responses in a wide range of allergic and inflammatory conditions (*6*). They proliferate after nematode infections and are involved in the eradication of intestinal worms (*7*). Two phenotypically distinct mast cell populations exist in the rat (*6, 8*). The thymus-independent connective tissue mast cells are concentrated in the serosal cavity and contain predominantly heparin and the enzyme rat mast cell protease I (RMCP I). The thymus-dependent mucosal mast cells are in the mucosal lamina propria of the intestine and lung and are characterized by the predominance of another proteoglycan, chondroitin sulfate di-B, and another enzyme, RMCP II, which is not found in other cells in the body (*6, 9*).

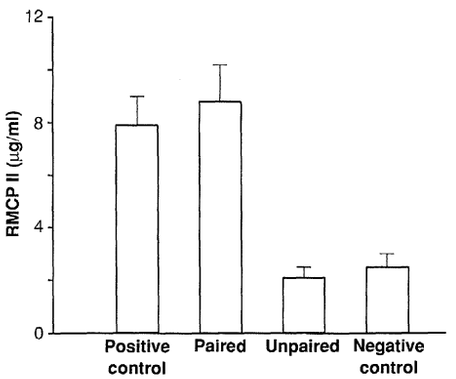

Fig. 1. Levels of RMCP II in serum 1 hour after reexposure to the CS. The values of RMCP II presented were obtained by subtracting baseline levels of RMCP II in serum (obtained 24 hours before challenge) from the levels of RMCP II found in serum at 1 hour. This within-animal manipulation eliminated the possibility that differences in RMCP II levels between groups before the challenge would affect differences in RMCP II levels after the challenge. Thus the presented values represent change from baseline levels of RMCP II in response to the CS challenge.

Table 1. Sensitization and conditioning protocol. Conditioned animals (paired and positive control groups) were exposed to the AV cue for 15 min in the conditioning chamber on days 35, 42, and 49, and then injected with egg albumin; the animals were then exposed to the AV cue for another 15 min. Another group of animals (unpaired) was placed in the chamber for 30 min on days 34, 41, and 48 and received the egg albumin in their home cages on the following day. All animals were left undisturbed for about 10 days and then reexposed to the CS on day 60. Conditioned animals were divided into two groups: (i) the paired group received only the CS at this time and (ii) the positive control group received the CS as well as the US. *n*, number of animals per group; NT, no treatment.

Group	*n*	Treatments		
		Days 34, 41, 48	Days 35, 42, 49	Day 60
Paired	10	NT	AV, EA	AV, PBS
Unpaired	11	AV	EA	AV, PBS
Negative control	11	NT	AV, PBS	AV, PBS
Positive control	11	NT	AV, EA	AV, EA

We developed an animal model of repeated antigen challenge on which we imposed a typical Pavlovian conditioning procedure (Table 1). On day 0, rats were sensitized with a subcutaneous injection of aluminum-precipitated egg albumin (1 ml of a 1 mg/ml solution) and an intraperitoneal injection of *Bordetella pertussis* (1 ml, 10^{10} organisms) (*10*). The sensitization protocol stimulates egg albumin–specific IgE; both the aluminum precipitate and the *Pertussis* injection act as adjuvants to promote IgE production (*11*). The animals were infected on day 14 with the larval form of the nematode *Nippostrongylus brasiliensis* to produce intestinal mast cell hyperplasia and promote IgE antibodies to egg albumin (*12*).

The CS was an audiovisual (AV) cue (*13*), and the US was a subcutaneous injection of egg albumin [300 μg in 0.6 ml of phosphate-buffered saline (PBS)]. The conditioning protocol described in Table 1 was followed. Blood was sampled 24 hours before the final reexposure to the CS on day 60, as well as at 1 and 5 hours after the exposure to the CS. Exposure of sensitized animals to egg albumin would be expected to cause mast cell stimulation and release of mediators. Thus the expected unconditional response to the egg albumin injections received during training would be release of these mediators. The experiment was designed to assess whether mucosal mast cells from paired group animals would now release mediators in response to exposure to the CS.

At 1 hour after challenge, RMCP II levels (*14*) differed significantly between groups [$F(3,39) = 15.58$, $P < 0.001$] (Fig. 1). The paired and unpaired group animals differed significantly in their response to the CS (mean ± SEM; 8.8 ± 1.4 μg/ml, paired; 2.1 ± 0.4 μg/ml, unpaired; Newman-Keuls, $P < 0.01$). The paired and unpaired groups differed during training only in the time interval between CS and US; thus the difference between the paired and unpaired groups represents the effect of conditioning. There was no statistically significant difference in the quantity of RMCP II released into the sera by either paired or positive control animals (8.8 ± 1.4 μg/ml and 7.9 ± 1.1 μg/ml, respectively). The CS, therefore, elicited a conditional response from paired group animals that was equivalent to the unconditional release of RMCP II (from positive control animals that received the egg albumin during the reexposure phase). Five hours after challenge, RMCP II in the sera of animals from all groups declined to approximately 1 μg/ml above baseline levels. Differences between groups were not significant [$F(3,39) = 1.55$, $P > 0.20$].

Our data indicate that the central nervous system can regulate the secretion of a mediator known to occur only in mucosal mast cells. These data support the increasing evidence for central nervous system involvement in the regulation of immune function (*3*). Our demonstration of conditional mast cell activity does not provide conclusive evidence for functional mast cell–nerve interactions; however, there is considerable evidence in vitro and in vivo to support both morphological and functional nerve–mast cell interactions (*15–17*).

Communication between mast cells and the nervous system may have a role in pathological intestinal conditions of uncertain etiology, such as Crohn's disease, ulcerative colitis, food sensitivity, and irritable bowel disease, where mucosal mast cell numbers are increased and psychological factors have been incriminated (*18*). In allergic diseases of the lungs and upper respiratory tract, such as allergic rhinitis and asthma, mucosal mast cells and psychological factors may also be of pathogenetic significance, although this has been disputed (*6, 19*). The conditional response we observed was equivalent to the unconditional response, suggesting that the conditional response was physiologically meaningful. Our results indicate that a consideration of mast cell–nervous system communication may be important for understanding and effectively treating a range of diseases.

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10. Male Sprague-Dawley rats were purchased from Charles River, and weighed between 225 to 250 g at the time of sensitization. Animals were provided with food and water ad libitum during the experiment and were maintained according to guidelines set forth by the Canadian Council on Animal Care.

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13. Animals were removed from the colony room and placed in plastic cages inside concrete-encased (soundproof) cabinets. A light flashed at an alteration rate of 300 ms, and background noise was provided by ventilation fans. The AV CS was based on that used by G. MacQueen and S. Siegel (*Behav. Neurosci.*, in press).
14. An enzyme-linked immunosorbent assay (ELISA) for detecting RMCP II was modified from Miller *et al.* (*7*). Rats were anesthetized with ether, and blood was obtained from the retro-orbital plexus. Sera were collected and stored at −20°C. The wells of a tissue culture microtitre plate (Nunclon Delta) were coated with 0.5 mg of RMCP II per milliliter of a 0.2*M* carbonate buffer, *p*H 9.6. Samples and standards were diluted in PBS containing 0.3% w/v bovine serum albumin, 0.02% v/v polyoxyethylene-sorbitan monolaurate (Tween 20), and 0.02% w/v sodium azide and incubated for 16 to 24 hours with a diluted specific rabbit antiserum to RMCP II (anti-RMCP II) from which all activity against RMCP I had been removed by immunoabsorption. After extensive washing of the plates, 100 μl of samples and standards were placed in duplicate wells and incubated for 16 to 24 hours. Plates were again washed several times and rabbit antibody bound to the plate was detected with an alkaline phosphatase conjugated goat to rabbit antibody (ICN) and a sodium-*p*-nitrophenyl phosphate substrate (Sigma). Results were calculated on the basis of a standard curve constructed with the use of known concentrations of purified RMCP II. Antibodies to RMCP II were raised in rabbits with purified RMCP II. Antisera were absorbed twice with RMCP I covalently attached to Sepharose 4B. The final preparation showed strong binding to RMCP II on immunoblot analysis, with a weak cross-reaction to RMCP I, and no detectable binding to cathepsin G. The specificity of this assay was confirmed with homogenates of tongue tissue known to contain high levels of RMCP I, but no RMCP II. Although there was some binding to RMCP I on the immunoblot, no binding was detected in the liquid phase.
15. Morphological studies have shown the presence of mast cells in peripheral nerves [Y. Olsson, *Acta. Neurol. Scand.* **47**, 357 (1971)] and autonomic ganglia [G. Gabella, *Structure of the Autonomic Nervous System* (Chapman and Hall, London, 1976)], a consistent ultrastructural relationship between mucosal mast cells and nerves in normal and nematode-infected rat lamina propria was detected [R. H. Stead, *Proc. Natl. Acad. Sci. U.S.A.* **84**, 2975 (1987)].
16. Substance P causes release of histamine from mast cells in vitro [F. Shanahan *et al.*, *J. Immunol.* **135**, 1331 (1985); O. Hagermark, T. Hockfelt, B. Pernow, *J. Invest. Dermatol.* **71**, 233 (1978)], and mast cells and substance P containing nerves may be involved in the vasodilatory response to noxious stimuli [J. C. Foreman and C. C. Jordan, *J. Physiol.* **238**, 58 (1982)].
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Structural Origins of High-Affinity Biotin Binding to Streptavidin

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The high affinity of the noncovalent interaction between biotin and streptavidin forms the basis for many diagnostic assays that require the formation of an irreversible and specific linkage between biological macromolecules. Comparison of the refined crystal structures of apo and a streptavidin:biotin complex shows that the high affinity results from several factors. These factors include the formation of multiple hydrogen bonds and van der Waals interactions between biotin and the protein, together with the ordering of surface polypeptide loops that bury the biotin in the protein interior. Structural alterations at the biotin binding site produce quaternary changes in the streptavidin tetramer. These changes apparently propagate through cooperative deformations in the twisted β sheets that link tetramer subunits.

STREPTAVIDIN IS A TETRAMERIC PROtein (molecular weight = 4 × 15,000) isolated from the actinobacterium *Streptomyces avidinii* (*1*). Streptavidin, and the homologous protein avidin, are remarkable for their ability to bind up to four molecules of *d*-biotin with unusually high affinity [dissociation constant $K_d = 10^{-15} M$ (*1, 2*)]. Although these proteins may function as antibiotics that deplete the environment of the essential vitamin biotin, they have been studied primarily as paradigms for understanding high-affinity protein-ligand interactions (*2*). At the same time, the ability of streptavidin and avidin to bind derivatized forms of biotin has led to their widespread use in diagnostic assays that require formation of an essentially irreversible and specific linkage between biological macromolecules (*3*). We undertook the structure determination of streptavidin, with and without bound biotin, to uncover the origins of high affinity of the protein for biotin.

Streptavidin was obtained from several commercial sources and produced different crystal forms during the course of the study. The most consistent results were obtained with a fragment of the native 159-residue streptavidin chain, incorporating residues 13 through 133. Numerous studies indicate that this truncated form of the molecule binds biotin with an affinity that is the same or similar to alternative longer versions of the protein. Moreover, in some cases it appeared that preparations identified as full-length material crystallized isomorphously with the truncated fragment, suggesting that the molecular terminii may be relatively flexible or disordered. Crystallization conditions for apostreptavidin and its biotin complex were found by robotic grid search methods (*4*). Both formed crystals from a polyethylene glycol–LiCl mixture, although the streptavidin:biotin complex crystallizes at *p*H 7.8 [space group $I4_122$, $a = b = 99.4$ Å, $c = 125.8$ Å (*5*)], whereas apostreptavidin crystallizes at *p*H 2.4 [space group $I4_122$, $a = b = 58.3$ Å, $c = 172.5$ Å]. Unit cell parameters of truncated apostreptavidin are similar to those reported by Pahler *et al.* (*6*), although the crystals studied here grow at lower *p*H, and diffract to higher resolution ($d_{min} = 1.7$ Å).

The structure of apostreptavidin was determined by multiple isomorphous replacement techniques. X-ray diffraction data for parent crystals and several isomorphous replacement derivatives were collected using a multiwire area detector and processed with the Xengen data-reduction package (*7*). Successful derivatives included $K_2Pt(SCN)_6$, which was prepared by soaking crystals in the heavy metal solution, and an iodine derivative prepared by crystallizing protein after reaction in solution (*8*). Substitution sites were located by an automated search procedure (*9*) performed on the heavy atom difference Patterson maps. Phases were ob-

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