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Sensitization of mice to chemical allergens modulates the responsiveness of isolated mast cells to IgE-dependent activation

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SUMMARY

It is known that the release of granule-associated inflammatory amines by isolated mouse tissue-type mast cells is subject to regulation *in vitro* by a number of cytokines that are produced during the immune response. In this study we investigated whether mast cell secretory function might also be subject to regulation *in vivo* during active sensitization. Mice were sensitized with one of three chemical allergens (trimellitic anhydride, TMA; 2,4-dinitrochlorobenzene, DNCB; or oxazolone) all of which induce contact sensitization and one of which (TMA) in addition causes immediate hypersensitivity. Peritoneal mast cells isolated from treated mice and sensitized passively with IgE released a greater proportion of cellular serotonin (5-HT) on stimulation either by anti-IgE or by specific antigen than did cells isolated from vehicle-treated controls. These results show that the function of mast cells is susceptible *in vivo* to regulatory influences that result from induction of an immune response.

The growth and differentiation of mast cells is known to be controlled by a number of cytokines derived from T lymphocytes and other sources. For example, the clonal growth of cultured mouse tissue-type mast cells is promoted by interleukin-3 (IL-3) acting in concert with IL-4[1] and the action of these two cytokines is antagonized by interferon-γ (IFN-γ).[2] A number of other cytokines synergize with IL-3 or IL-4, whereas the *c-kit* ligand, also known as stem cell factor (SCF), is on its own a potent promotor of mast cell growth.[3] Recently we found that SCF, IL-3 or IL-4 enhance the functional responsiveness of isolated mouse tissue-type mast cells to stimulation with specific IgE and antigen, measured as release of the granule-associated mediator serotonin.[4] As had been shown for mast cell growth,[2] the secretory responsiveness of mouse mast cells to activation via IgE/antigen and to a range of other stimuli is down-regulated by IFN-γ.[5] Since IL-3, IL-4 and IFN-γ are generated by T lymphocytes during the induction phase of immune responses, we questioned whether active immunization might result in modulation of mast cell function *in vivo*. To this end, we selected as immunogens certain chemical allergens, all of which we have shown to induce T-cell activation in draining lymph nodes in mice, but which provoke different patterns of antibody responses.[6,7]

Young adult (8–12 weeks) female BALB/c strain mice (Barriered Animal Breeding Unit, Alderley Park, U.K.) were immunized topically with 50 μl of chemical on both planks on days 0 and 6 and 25 μl of the same chemical on the dorsum of both ears on day 3. The following chemicals were used as solutions in 4:1 acetone:olive oil (AOO): 50% trimellitic anhydride (TMA; Aldrich Chemical Co., Gillingham, U.K.), 5% methyl salicylate (Aldrich), 1% 2,4-dinitrochlorobenzene (DNCB) and 1% 4-ethoxymethylene-2-phenoxazol-5-one (oxazolone; Sigma Chemical Co., St Louis, MO). Doses of TMA, DNCB and oxazolone were selected as those that we have found previously to induce proliferative responses of comparable vigour in draining lymph nodes.[6,7] In contrast, methyl salicylate is non-allergenic and fails to cause lymph node activation.[8] In each experiment, mice ($n=3$) received chemical or vehicle (AOO) alone. Three weeks following initiation of exposure, peritoneal cells were isolated by lavage with RPMI-1640 medium (Gibco, Paisley, U.K.). Cells were pooled for each experimental group, sedimented (150 ***g***, 5 min), washed once with RPMI-1640 and resuspended in RPMI supplemented with 2 mM L-glutamine, 40 μg/ml gentamycin and 5% foetal calf serum (RPMI-FCS). On the basis of metachromatic staining in 0·01% aqueous toluidine blue, mast cells comprised between 1 and 2% of the peritoneal mast cell population. The cells were adjusted with RPMI-FCS to provide 10^4 mast cells/ml and were cultured in 1-ml volumes with 1 μg/ml monoclonal mouse IgE anti-DNP antibody (ICN Flow, High Wycombe, U.K.) in 24-well culture plates for 24 hr. For the final hour of incubation 5-[1,2-^{3}H(N)]-hydroxytryptamine creatinine sulphate ([^{3}H]5-HT, NEN, Dreieich, Germany, sp. act. 27 Ci/mmol) was added to the cells (1·0 μCi/ml, 37 kBq/ml). [^{3}H]5-HT is taken up selectively by rodent mast cell granules and is released in parallel with endogenous mediators such as histamine, thus acting as a specific marker of mast cell degranulation. At the termination of

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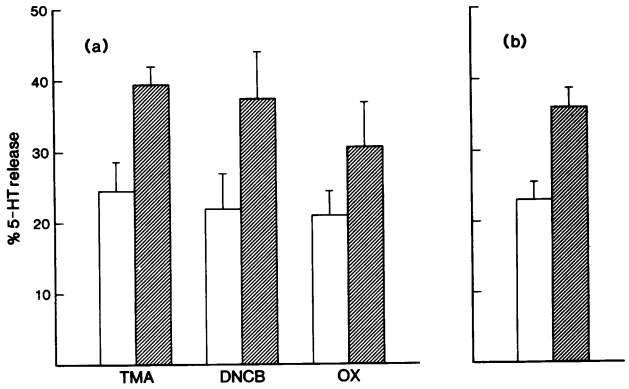

Figure 1. Effect of topical exposure of mice to chemical allergens on serotonin release from isolated mast cells stimulated with IgE/anti-IgE. Groups of mice ($n=3$) received TMA (50%), DNCB (1%), oxazolone (1%) or vehicle alone (AOO) as described in the text. (a) Results for individual allergens (TMA, $n=8$; DNCB, $n=5$; oxazolone, $n=6$). (b) Results for pooled allergens ($n=19$). Data are expressed as means +SEM. Data from mice exposed to chemical allergen (■) and mice exposed to AOO control (□). For each comparison of data from chemical-treated versus control-treated mice, $P<0.05$ by two-tailed paired Student's t-test.

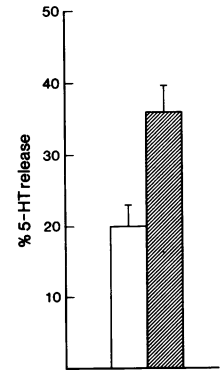

Figure 2. Effect of topical exposure of mice to chemical allergens on serotonin release from isolated mast cells stimulated with IgE/antigen. Data from mice exposed to chemical allergen (■) and mice exposed to AOO control (□). Results were pooled from a total of six experiments (each of the three chemicals was tested on two separate occasions) and are expressed as means + SEM. For comparison of data from chemical-treated versus control-treated mice, $P<0.005$ by two-tailed paired Student's t-test.

cultures the cells were aspirated from the culture plate wells into RPMI containing 1% FCS, washed twice and resuspended in 1·2 ml of RPMI-FCS supplemented with 10 mM HEPES to give pH 7·0 at 37°. Cells (150 μl) were challenged in duplicate for 10 min at 37° with an equal volume of prewarmed (37°) challenge medium containing either rat monoclonal anti-mouse IgE antibody (0·1 μg/ml; kindly provided by Dr F. D. Finkelman, Uniformed Services University of the Health Sciences, Bethesda, MD) or DNP_{13}–HSA conjugate (1·0 μg/ml). The concentration of each of these agents was selected to give optimal release of 5-HT from mast cells bearing IgE antibody. Cells were added simultaneously to equal volumes of prewarmed medium alone as control or 0·05% Triton-X to lyse the cells. At the end of challenge the cells were sedimented rapidly (200 *g* 2 min) and 150 μl of supernatant fraction removed for β-scintillation counting. Specific stimulated 5-HT release was calculated as $[(a-b)/c] \times 100$ where a = stimulated release, b = control release, and c = total cell content.

Mast cells isolated from mice sensitized to TMA, DNCB and oxazolone released significantly greater levels of 5-HT compared with cells obtained from vehicle (AOO)-treated control mice upon incubation with IgE antibody and subsequent challenge with anti-IgE (Fig. 1). We have demonstrated previously that exposure of mice to TMA under similar conditions to those used in the present study leads to an elevation of total serum IgE[6] and also to the production of specific IgE antibody.[6] However, DNCB and oxazolone both fail to elevate serum IgE or to induce specific IgE.[6,7] Thus, although in the case of TMA elevated responsiveness to anti-IgE could be explained by an increase in the amount of cell surface receptor-bound IgE, with both DNCB and oxazolone this cannot be the case. We conclude that at least for the latter two compounds, treatment of the mice has resulted in an *in vivo* modulation of mast cell responsiveness to IgE-dependent stimulation. This conclusion is confirmed by experiments in which mast cells isolated from allergen-exposed mice were incubated with IgE antibody and challenged with antigen (DNP). As the data summarized in Fig. 2 show, mast cells obtained from mice exposed to the chemical allergens TMA, DNCB or oxazolone, manifested increased responsiveness to this mode of specific stimulation. No increase in mast cell responsiveness to IgE combined with anti-IgE or antigen was seen when mice were exposed to the non-allergenic chemical methyl salicylate (data not shown). The elevated responsiveness to activation by IgE/antigen of cells from mice exposed to TMA and oxazolone, as shown in Fig 2, is clearly not attributable to an *in vivo* IgE response, since any elevations in total or specific IgE on cell surface receptors would actually deter binding of an unrelated specific IgE antibody *in vitro*. In the case of mice sensitized to DNCB, an increase in cell surface IgE anti-DNP could have increased responsiveness to DNP-HSA antigen, but as we have previously shown that DNCB fails to induce an IgE response in mice[6] this explanation is untenable. We conclude therefore that sensitization of mice to TMA and oxazolone, and probably also DNCB, leads to an up-regulation of mast cell function *in vivo* that is unrelated to induction of IgE antibody. The absence of any modulatory activity *in vivo* of methyl salicylate is consistent with the known inability of this chemical to induce an immune response in mice, and reinforces the link between immune response and regulation of mast cell function that we see with allergenic chemicals.

TMA is a known human respiratory allergen. Although TMA, DNCB and oxazolone each induce in mice contact sensitization and IgG anti-hapten antibody, exposure only to TMA results in an IgE antibody response.[6,7] We have speculated previously that the qualitative differences in the immune responses provoked in mice by contact and respiratory allergens reflects preferential activation of Th1- and Th2-type responses respectively.[6] We have found also that IL-4 (a product of Th2 cells)[9] or IL-3 (a product of both Th1 and Th2 cells)[9] each enhance mast cell secretory function *in vitro*,[4] whereas IFN-γ (a Th1 cell product)[9] inhibits secretory function.[5] A predominance of IL-4 over IFN-γ synthesis in mice sensitized to respiratory chemicals would be predicted with the converse in mice exposed to contact allergens which lack the potential to induce respiratory hypersensitivity. However, in the present investigation

exposure of mice to either TMA or to DNCB/oxazolone resulted in a comparable degree of promotion of mast cell function, suggesting perhaps that *in vivo* these treatments induce a similar proportion of cytokines that up-regulate mast cell function (perhaps a combination of IL-3 and IL-4).

In this report we have shown that the secretory responsiveness of isolated tissue-type mast cells to IgE and anti-IgE/antigen can be influenced *in vivo* by sensitization of mice to chemical allergens independently of an IgE response. These findings show that regulation of mast cell function occurs as an integral component of at least certain kinds of immune response. The regulatory effects on secretion of IL-3 and IL-4 have been demonstrated previously *in vitro*, and these or related cytokines may be important in natural processes of regulation of mast cell function *in vivo*.

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