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Tolerance to experimental contact sensitivity induced by T cell vaccination

It was shown previously that experimental autoimmune diseases could be prevented or treated specifically by administering suitably attenuated autoimmune T lymphocytes to animals, a process termed T cell vaccination (Cohen, I. R., *Sci. American* 1988. *256:* 52). We now report that T cell vaccination is an effective way of inducing tolerance to contact sensitivity to simple chemical haptens. Vaccines were prepared from populations of lymph node cells from specifically sensitized mice by activating the T cells with the T cell mitogen concanavalin A and then treating the T cell blasts with glutaraldehyde. The vaccinated mice showed decreased delayed sensitivity responses to the specific sensitizing antigen and developed significant delayed sensitivity responses to the T cells of the same specificity as those used for vaccination. Thus, T cell vaccination against contact sensitivity reactions appears to function similarly to T cell vaccination against autoimmune disease.

1 Introduction

Contact sensitivity (CS) is an example of antigen-specific DTH. CS reactions are caused by clones of effector T lymphocytes that recognize chemical antigens in the context of class II MHC gene products in the skin [1, 2]. Accordingly, it should be possible to regulate CS reactions by inducing an immune response directed against the effector T cell clones specific for the CS antigen. This strategy was used to regulate experimental autoimmune disease using as vaccines lines and clones of autoimmune T_h lymphocytes capable of causing various autoimmune diseases in rats or mice [3–5]. It was observed that some attenuated autoimmune lymphocytes could be used to induce resistance to specific autoimmune diseases produced by virulent autoimmune T lymphocytes of similar specificity [6, 7]. This maneuver was termed T cell vaccination.

Recently, it was discovered that the effectiveness of T cell vaccination could be augmented by treating autoimmune T cells with chemical cross-linkers such as glutaraldehyde which is known to cause chemical aggregation of membrane components [8].

In this present report, we describe a model system for inducing tolerance to CS using vaccines consisting of glutaraldehyde-treated immune LN cells (I-LNC). Vaccination with T cells caused a significant (>50%) antigen-specific inhibition of CS associated with a specific response to the T cell vaccine.

2 Materials and methods

2.1 Mice and reagents

BALB/c female mice were purchased from the Jackson Laboratories (Bar Harbor, ME). All mice were used at 8–12 weeks of age. 2,4-Dinitro-1-fluorobenzene (DNFB) and 2,4-dinitrobenzene sulfonic acid sodium salt ($DNBSO_3^-$) were obtained from Eastman Kodak Co. (Rochester, NY). 4-Ethoxymethylene-2-phenyl oxazolone (Ox) was purchased from BDH Chemicals Ltd. (Poole, GB). Picryl chloride (PiCl) and glutaraldehyde (GA) were obtained from Fluka (Buchs, Switzerland).

2.2 Sensitization

Groups containing at least four mice were sensitized on the shaved abdominal skin with either 25 μl of 0.5% DNFB or 25 μl of 2% Ox in a vehicle of 4 : 1 (v : v) acetone : olive oil. Sensitization for PiCl was performed with 75 μl of 5% of the hapten in the same diluent. Double sensitization with DNFB and Ox was done by painting the right side of the abdomen with Ox and the left side of the abdomen with DNFB, leaving a strip of unshaven abdomen in the center.

2.3 Elicitation of contact sensitivity

Mice were ear challenged with either 20 μl of 0.2% DNFB or 20 μl of 0.5% Ox (10 μl to each side of the ear) in the same vehicle as described above. Elicitation of CS to PiCl was performed with 10 μl of 1% (5 μl to each side of the ear) in olive oil. A constant area on the ears was measured immediately before challenge and 24 h later with a Mitutoyo engineer's micrometer (Mituyoto, Tokyo, Japan). The CS reaction was determined as the amount of swelling, the difference between the two measurements expressed as the mean in units of 10^{-4} inch or 10^{-2} mm (depending on the

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Abbreviations: **CS:** Contact sensitivity **DNFB:** 2,4-Dinitro-1-fluorobenzene **$DNBSO_3^-$:** 2,4-Dinitrobenzene sulfonic acid **GA:** Glutaraldehyde **I-LNC:** Immune lymph node cells **Ox:** Oxazolone **PiCl:** Picryl chloride

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micrometer used) ± SE. Percent inhibition was calculated as

$$\% \text{ Inhibition} = \frac{\text{positive control} - \text{experimental group}}{\text{positive control}} \times 100$$

In all calculations of inhibition, background ear swelling (irritation effect) caused by the hapten in unsensitized mice was subtracted from the other groups [9].

2.4 Transfer of contact sensitivity

DNFB DNP-I-LNC were obtained from donor mice sensitized on day 0 and 1 with a total of 55 μl of 0.5% DNFB in 4 : 1 acetone : olive oil (25 μl applied to the shaved abdomen and 5 μl to each of the feet and ears). I-LNC (Ox) were obtained from donor mice sensitized with a total of 80 μl 2% Ox in 4 : 1 acetone : olive oil (50 μl of the abdomen and 5 μl to each of the feet and ears). On day 4 (for DNFB) or day 5 (for Ox) the draining LN were removed and single cell suspensions were prepared and washed in RPMI 1640 medium. Recipients were given 5×10^7 I-LNC i.v. Mice were ear challenged within 1 h of transfer. For cotransfer experiments, I-LNC from two different donor groups were mixed (1 : 1) and injected i.v. into the same mouse [10].

2.5 T lymphocyte vaccination

I-LNC, obtained from donor mice as described above, were resuspended (3×10^6–5×10^6 cells/ml) in Dulbecco's modified Eagle's medium (Gibco, Grand Island, NY), supplemented with L-glutamine (1 mM), 2-ME (5×10^{-5} M) and 1% autologous mouse serum. Con A (1.2 μg/ml, Yeda, Rehovot, Israel) was added to activate the T lymphocytes since non-activated T cells do not vaccinate [7]. At the end of 48-h incubation (37 °C, 5% CO_2) the cells were washed twice with PBS, pelleted and resuspended in 3 ml PBS (10^8 cells). An equal volume of 0.6% GA in PBS was then added and incubated at room temperature for 15 min. The treated I-LNC were washed 4–5 times by centrifugation in 50 ml of PBS and injected i.p. into recipient mice (2×10^7–3×10^7 cells/0.5 ml/mouse). In several experiments control vaccines consisted of Con A-treated irradiated (2500 rad) I-LNC which were injected i.p. as described above.

2.6 *In vitro* antigen stimulation

Draining LN were removed and single-cell suspensions were obtained by pressing the LN through stainless steel screens. Cells were washed twice and resuspended in RPMI 1640. Then 1×10^5 viable cells were cultured in round-bottom, 96-well culture plates (Greiner, Nürtingen, FRG) containing 0.2 ml RPMI 1640 supplemented with L-glutamine (0.3 mg/ml), penicillin (100 U/ml), streptomycin (100 μg/ml) and 5% heat-inactivated FCS. The cells were cultured for 72 h at 37 °C in a humidified atmosphere of 5% CO_2 in the presence (2.5 μg/ml) or absence of $DNBSO_3^-$ hapten. Cultures were pulsed with 1 μCi = 37 kBq [^{3}H]dThd for the last 18 h of incubation before harvesting with an automated sample harvester. The results of triplicate cultures are expressed as cpm ± SEM or stimulation index (SI):

$$SI = \frac{\text{cpm of cells} + DNBSO_3^-}{\text{cpm of cells} - DNBSO_3^-}$$

2.7 Statistical analysis

Student's two-tailed *t*-test was used to determine the statistical significance between experimental and control groups. A $p < 0.05$ was considered to be significant.

3 Results

3.1 Tolerance induction by GA-treated T lymphocytes

Figs. 1 and 2 summarize the effects of vaccinating mice with GA-treated I-LNC on the development of actively induced contact sensitivity to two antigens, DNFB and Ox. Vaccinating mice with GA-treated I-LNC (DNFB) 7 days prior to sensitization caused 50% suppression of the response to DNFB as compared to that of unvaccinated mice (Fig. 1, group D *vs.* A, $p < 0.002$). Similarly, 73% suppression of the contact sensitivity response to Ox was caused by

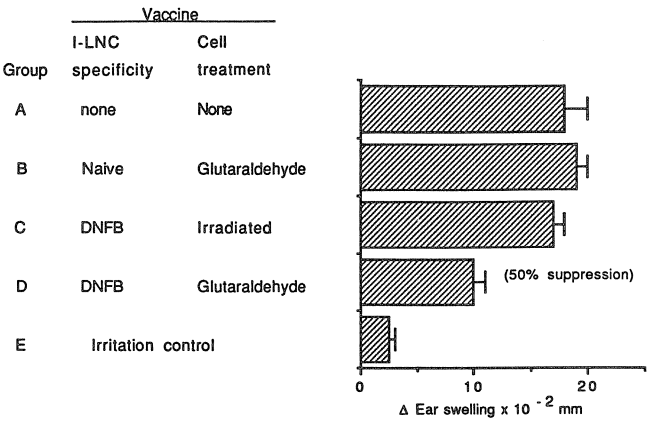

Figure 1. T cell vaccination against CS to DNFB. Mice were vaccinated i.p. with Con A-activated irradiated I-LNC (group C) or with Con A-activated GA-treated I-LNC (group D). One group received Con A-activated GA-treated naive LNC (group B). Seven days later, these mice were epicutaneously sensitized (on the shaved abdomen) with DNFB. All mice were ear challenged 5 days post sensitization and ear swelling was measured 24 h later. Fifty percent suppression of the contact response was observed only in mice treated with GA-modified lymphocytes.

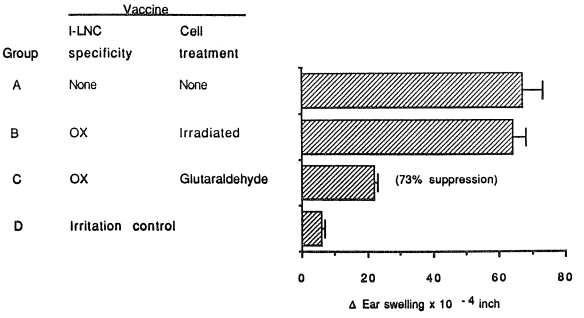

Figure 2. T cell vaccination against CS to Ox. The experiment was conducted as described in Fig. 1; however, both donors and recipients of the I-LNC were sensitized with Ox.

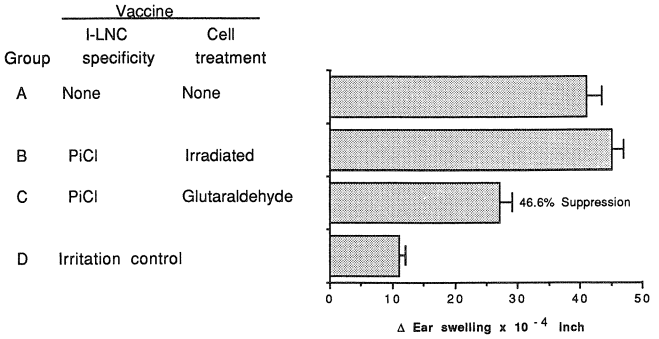

Figure 3. T cell vaccination against CS to PiCl. The experiment was conducted as described in Fig. 1; however, both donors and recipients of the I-LNC were sensitized with PiCl.

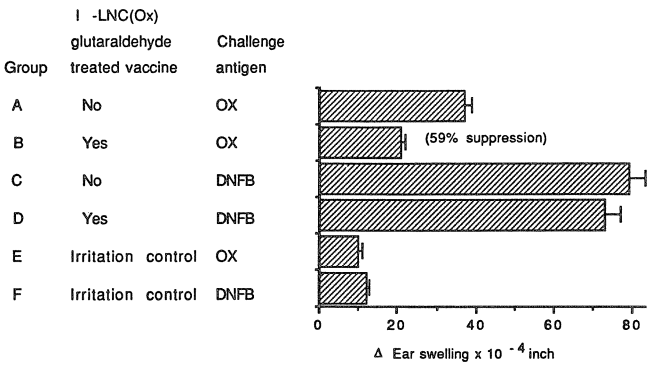

Figure 4. Specificity of vaccination (active sensitization). Mice received simultaneous sensitization with both Ox and DNFB. Vaccination was induced (1 week prior to sensitization) by Con A-activated GA-treated I-LNC obtained from Ox-sensitized donor mice [I-LNC (Ox)]. Significant inhibition of the contact response was achieved only in mice challenged with Ox (group B).

vaccination with GA-treated I-LNC (Ox) (Fig. 2, group C *vs.* A, $p < 0.001$). Two different control groups were included in these experiments: mice that had been vaccinated with irradiated I-LNC (groups C and B in Figs. 1 and 2, respectively) and mice that had been vaccinated with GA-treated *naive* LNC (Fig. 1, group B). Neither of these treatments affected the development of CS. Similarly a 47% inhibition of CS was found in PiCl-sensitized mice which had been vaccinated (7 days prior to sensitization with Con A-activated GA-treated I-LNC (Fig. 3). Thus significant tolerance to CS could be achieved by vaccination with

Table 1. The effect of T cell vaccination on proliferative responses *in vitro*[a)]

Donor mice vaccination	Proliferative response (cpm ± SEM)	
	without $DNBSO_3$	with $DNBSO_3$
None	2676 ± 342	12 203 ± 295 (4.6)
I-LNC (irrad.)	1781 ± 235	10 293 ± 811 (5.8)
I-LNC (GA-treated)	2635 ± 301	6 675 ± 158 (2.5)

a) Naive or vaccinated mice (day 7) were sensitized with DNFB on days 0 and 1. LNC were harvested on day 5 and were studied for a proliferative response to $DNBSO_3^-$. Numbers in parenthesis indicate the stimulation index.

GA-treated I-LNC. This tolerance could not be attributed to antigen carry-over since irradiated I-LNC did not have any significant effect on the contact response.

As in other antigen-specific systems, lymphoid cells from mice contact sensitized to DNFB show antigen-specific T cell proliferation [1]. Therefore, we wished to see if *in vivo* tolerance to DNFB induced by T cell vaccination affected *in vitro* proliferative responses to $DNBSO_3^-$. The results are shown in Table 1. I-LNC from sensitized untreated mice gave a significant proliferative response to $DNBSO_3^-$ (stimulation indices of about 5). I-LNC obtained from mice that had been vaccinated with irradiated I-LNC(DNFB) showed a proliferative response which was not different from the response seen in lymphocytes from untreated immunized mice. However, a significant reduction in the proliferative response was observed when the mice had been vaccinated with GA-treated I-LNC(DNFB) ($p < 0.01$).

3.2 Specificity of vaccination

Two types of experiments were conducted to test the antigen specificity of T cell vaccination against CS. First, we vaccinated mice with GA-treated I-LNC (Ox), and 7 days later sensitized them with a combination of Ox and DNFB. As shown in Fig. 4, mice vaccinated with GA-treated I-LNC (Ox) and later sensitized with both Ox and DNFB developed significant inhibition (59%) of the contact response to Ox (groups B *vs.* A, $p < 0.002$), but not to DNFB (group C *vs.* D, not significant). As before, irradiated I-LNC failed to vaccinate.

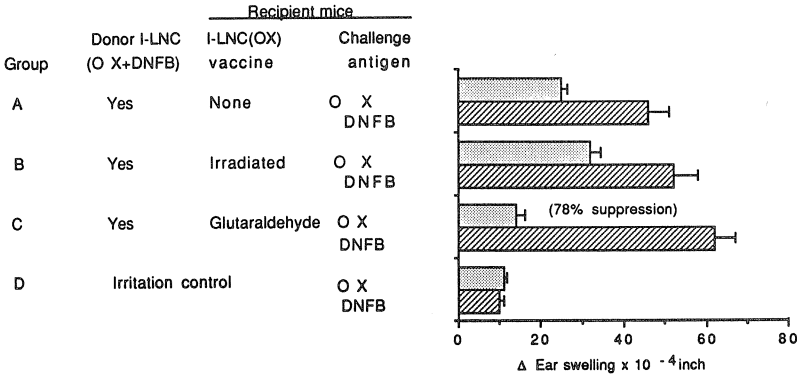

Figure 5. Specificity of vaccination (adoptive sensitization). Vaccinated or unvaccinated mice were adoptively sensitized with mixed (1 : 1) populations of I-LNC (Ox) plus I-LNC (DNFB). The left ears of the recipients were challenged with Ox and the right ones with DNFB. Seventy-eight percent suppression was observed only in the left ears of mice vaccinated with Con A-activated GA-treated I-LNC (Ox).

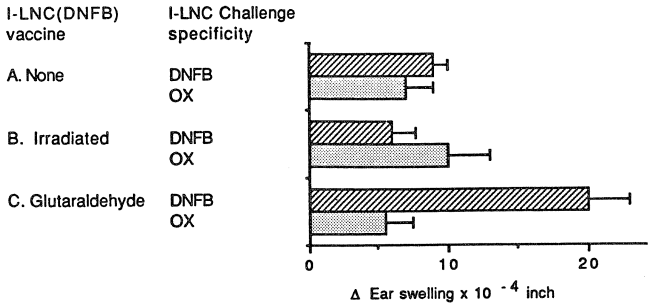

Figure 6. Anti-idiotypic DTH reactivity to the vaccine. Mice were vaccinated i.p. with Con A-activated GA-treated I-LNC (DNFB) (group C) or with Con A-activated irradiated I-LNC (group B). One week later the mice were epicutaneously sensitized with DNFB on the shaved abdomen. Five days after sensitization, DTH was measured as the increase in ear swelling after 24 h in response to Con A-activated irradiated (2500 rad) I-LNC which had been injected intradermally into the ears.

In the second set of experiments, of which one representative is shown in Fig. 5, we analyzed the effect of vaccination against CS to one antigen (DNFB) on adoptive transfer of contact sensitivity to two antigens (DNFB and Ox). I-LNC (DNFB) and I-LNC (Ox) were transferred together into recipient mice that had been vaccinated one week before transfer with GA-treated I-LNC (Ox), or left unvaccinated (groups C and A, respectively). The left ears of the recipients were then challenged with DNFB and the right ears with Ox. Mice that had been vaccinated with GA-treated I-LNC (Ox) exhibited significant suppression of the response to Ox (78%; C *vs.* A for Ox, $p < 0.001$). By contrast, there was no detectable inhibition of the response to the DNFB (compare groups C and A). Once again, it was found that vaccinating the recipient mice with irradiated I-LNC did not cause any significant change in the contact response to either the relevant (Ox) or irrelevant antigen (DNFB). Thus T cell vaccination induced immunologically specific inhibition of CS produced either by active immunization or by passive transfer of I-LNC.

3.3 Anti-idiotypic DTH to the T cell vaccine

As the antigen sensitivity of the vaccinating T cells influenced the sensitivity of the tolerance to CS, it was conceivable that vaccination might involve the induction of immunity to the antigen receptors of the vaccines [7, 12, 13].

To test this hypothesis we investigated the DTH response of vaccinated mice to the specific I-LNC. Fig. 6 shows the results of an experiment in which mice were or were not vaccinated with GA-treated I-LNC (DNFB). Seven days later these animals were sensitized with DNFB and their responses were measured to Con A-activated, irradiated (2500 rad) I-LNC (DNFB) or I-LNC (Ox) injected i.d. into the ears 5 days after sensitization.

Non-vaccinated control mice (group A) showed background ear swelling to either I-LNC (Ox) or I-LNC (DNFB). No significant difference, compared to the background response, was found when mice were treated with irradiated I-LNC (DNFB) (group B *vs.* A, not significant for both ears). In contrast mice vaccinated with GA-treated I-LNC (DNFB) (group C), which were found to be resistant to contact sensitivity to DNFB (not shown), manifested a specific DTH response to I-LNC (DNFB) and no significant reaction to I-LNC (Ox) in the contralateral ear ($p < 0.05$). It may therefore be concluded that the tolerance to CS in the vaccinated mice is associated with a T cell response specific for the vaccinating T cells.

4 Discussion

T cell vaccination was originally demonstrated to be effective against a variety of experimental autoimmune diseases using antigen-specific lines or clones of T lymphocytes attenuated by irradiation [5–8]. Rats or mice receiving irradiated T lymphocytes reactive to myelin basic protein or to thyroglobulin acquired resistance to subsequent attempts to induce EAE [6] or thyroiditis [5]. Vaccination against adjuvant arthritis could be achieved using an antigen-specific T cell line that did not have to be attenuated by irradiation [14]. It was later discovered that some virulent clones of T cells could induce resistance without causing disease when administered at cell doses below the threshold for transfer of disease (10^4 or fewer cells) [13, 15, 16].

A prerequisite for effective vaccination, as for transfer of disease, is activation of the T cells before administration [17]. T cells will neither cause disease nor induce resistance if they have not been cultured with their specific antigen or with a T cell mitogen such as Con A before being administered to recipient animals. Activation, among other effects produces changes in T cell membrane components, modifies T cell traffic in the body [17, 18] and induces expression of enzymes [19]. However, the elements of activation critical for T cell vaccination are yet to be defined.

It was found that the capacity of activated T cells to vaccinate could be greatly enhanced by treating them in ways that caused aggregation of membrane components [7, 8]. A rat T cell clone that otherwise could not vaccinate, upon treatment with hydrostatic pressure or the chemical cross-linker GA acquired the capacity to prevent adjuvant arthritis and to induce rapid remission of already established disease [8]. Agents such as GA were also able to augment the ability of uncloned populations of LN T cells from antigen-primed animals to vaccinate [8].

The mechanism of resistance induced by T cell vaccination seems to involve anti-idiotypic T cells arising in the vaccinated animal [13]. T cells of both CD4 and CD8 phenotypes obtained from the disease-resistant animals responded specifically to the T cell clone used for vaccination. Indeed, a CD8 T cell clone responsive to an anti-basic protein T cell was able to mediate resistance to adoptive transfer of encephalomyelitis [20]. However, protective mechanisms other than anti-idiotypic T cells are probably also set into motion by T cell vaccination [21].

The present study extends T cell vaccination beyond the area of autoimmunity to include contact sensitivity to defined chemicals. It could be argued that T cell vaccination is effective in autoimmunity because anti-idiotypic networks may arise naturally during differentiation of the

immune system as a safeguard against autoimmune disease [22]. Thus, vaccination using self-reactive T cells might exploit and strengthen an existing network. The present results indicate that T cell vaccination may also be tolerogenic for simple chemicals such as DNFB and Ox. Nevertheless, these chemicals are haptens and acquire immunogenicity through combination with carrier molecules that are self antigens themselves [1]. Thus contact sensitivity viewed as a form of modified self may be closely related to true autoimmunity.

In addition to inducing decreased reactivity to skin-sensitizing antigen, both *in vivo* and *in vitro*, T cell vaccination appeared to cause specific DTH to the antigen-specific population of I-LNC used for vaccination. This is compatible with a T cell response to the antigen-specific T cells mediating CS, a form of anti-idiotypic immunity. This hypothesis will be tested when T cell clones specific for DNFB or Ox become available.

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