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T helper factor in contact sensitivity: antigen-specific I-A$^+$ helper factor is made by an Lyt-1$^+$2$^-$, I-A$^+$, I-J$^-$ T cell

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Summary. Antigen-specific T helper factor appears in the 24 hr supernatant of lymph node cells taken 4 days after immunization with contact sensitizer. The factor is assayed by its ability to augment the contact sensitivity response induced by haptenized spleen cells. In practice, picrylated or oxazolonated spleen cells are treated with the factor for 1 hr at 4° and 4×10^6 cells are injected into the footpads of recipient mice. Contact sensitivity is assessed 5 days later.

The factor first appears 3 days after immunization and its production depends on an Lyt-1$^+$2$^-$, I-A$^+$, I-J$^-$ T cell. It is antigen-specific in its action in a criss-cross experiment, and can be absorbed with and eluted from haptenized beads. It bears I-A determinant(s) and the I-A determinant and the antigen binding site(s) occur on the same molecule. The molecular weight is around 60,000. The possible role of T helper factors in the activation of the antigen-presenting cell in the induction stage of the immune response is discussed.

INTRODUCTION

Both T suppressor and T helper cells and the corresponding antigen-specific factors have been described in antibody systems (Taussig, 1980; Altman & Katz, 1980). Similarly there is considerable literature on T suppressor cells and antigen-specific factors in delayed and contact hypersensitivity and several reports of T helper cells (Bretscher, 1979; Leung & Ada, 1981; Tucker & Bretscher, 1982; Miller & Butler, 1983; Wright & Ramshaw, 1983). However, the corresponding antigen-specific T helper factor has not been described. For this reason, we looked for T helper cells and T helper factor in contact sensitivity. The simple experiment of examining the helper cell function of 4 day immune lymph node cells was complicated by the fact that 4 day immune cells induced contact sensitivity when injected alone. This immunization was due to IgM–hapten complexes on the surface of the cells and could be prevented by incubating the cells in complement (Asherson, Colizzi & Watkins, 1983). After this treatment T helper cell activity could be demonstrated, but its effect was antigen non-specific and H-2-unrestricted, perhaps because other cells in the population produced non-specific lymphokines. However, the supernatant of these cells contained helper factor. This paper shows that the supernatant of 4 day immune cells contains antigen-specific T helper factor which bears I-A determinants and is made by an Lyt-1$^+$, I-A$^-$, I-J$^+$ T cell.

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MATERIALS AND METHODS

Production of T helper factor

Mice were immunized by applying 0·15 ml 5% picryl chloride or 3% oxazolone (4-ethoxymethylene-2-

phenyloxazolone) in alcohol to the clipped abdomen and thorax and four paws. The regional lymph nodes were harvested 4 days later. The T cells were purified by nylon wool and the cells cultured at 10^7/ml in RPMI-1640 with 2·5% inactivated foetal calf serum, added glutamine, penicillin and streptomycin. The supernatant was taken at 24 hr and was stored at −20°. Preliminary experiments showed that spleen cells did not produce active supernatants.

Assay of T helper factor

Picrylated (Pic) or oxazolonated (Ox), spleen cells were prepared by lysis of red cells with Boyle's solution followed by incubation in picrylsulphonic acid (1 mM, pH 7·2, room temperature, 10 min) or oxazolone (Zembala *et al.*, 1982) and then washed three times in phosphate-buffered saline. The haptenized cells were treated with the presumptive ThF supernatant (10^7 ml^{-1}, 4°, 1 hr), washed once and a total of 4×10^6 cells injected into both hind footpads of groups of four or five naive recipients. These mice were tested for contact sensitivity 5 days later by applying 1% oxazolone or picryl chloride to both sides of both ears. The ear swelling was measured after 24 hr with an engineer's micrometer and expressed in units of 10^{-3} cm ± standard deviation. Significance was assessed by Student's two-tailed *t*-test.

Absorption of T helper factor

Microcrystalline cellulose was activated with potassium periodate, coupled with diaminohexane and reduced with sodium borohydride. Finally, the free amino groups were derivatized with picrylsulphonic acid or 'oxazolone' (Asherson, Zembala & Noworolski, 1978). T helper factor (5 ml) was absorbed with 50 μl (wet) haptenized microcrystalline cellulose at room temperature for 30 min and eluted twice with 3 M sodium thiocyanate (2·5 ml, 30 min). Foetal calf serum was added to 1% to limit losses on surfaces and the thiocyanate removed by dialysis. Alternatively, haptenized red cells were used (10^8 ml^{-1}, 4°, 1 hr) for absorption.

Monoclonal anti-I-A^k (D2-3/F2a) or anti-I-A^b antibody was attached to sepharose activated with cyanogen bromide and made into a column for absorption and elution.

Cell separation

Four day immune nylon wool purified T cells were treated with monoclonal or conventional antibodies. Anti-Thy-1.2 was from OLAC, anti-I-A^k (D-2-3/F2a) from Dr B. E. Loveland and Dr. D. Poole and anti-Lyt-1.1 and Lyt-2.1 from Prof. I.F.C. McKenzie. The anti-I-J^k B10.A (3R) anti-5R serum has already been described (Zembala *et al.*, 1982; Malkovsky *et al.*, 1983). After treatment with antibody at 4° for 30 min at 5×10^8 ml^{-1}, the cells were washed once and separated into marker positive and marker negative subpopulations by 'panning' on bacteriological petri-dishes coated with affinity purified rabbit $F(ab')_2$ anti-mouse Ig (Zembala *et al.*, 1982). The cells were then cultured at 10^7 ml^{-1} for 48 hr and the supernatant tested for T helper factor activity. Killing of cells by antibody and complement follows Malkovsky *et al.* (1983).

Gel filtration

T helper factor (16 ml) was concentrated to 5·5 ml on an Amicon YM10 membrane. This was applied to a 3×90 cm column of Sephadex G-100 and eluted at 10 ml h^{-1}. Fractions of 5 ml were pooled in molecular weight bands of 10,000 and concentrated to 3 ml using blue dextran, bovine serum albumin, ovalbumin, chymotrypsinogen and cytochrome C as markers.

RESULTS

Antigen-specific T helper factor

The assay for T helper factor (ThF) was based on the augmentation of the contact sensitivity response to suboptimal numbers of lightly picrylated spleen cells. The helper factor was the 24 hr supernatant of 4 day, immune nylon T cells and the picrylated cells used for immunization were treated with helper factor at 4° for 1 hr, spun down and then injected into the footpads. Contact sensitivity was assessed 5 days later and measured in units of 10^{-3} cm ± standard deviation. The top two lines of Fig. 1 show that an adequate number of heavily picrylated spleen cells gave rise to contact sensitivity and there was no additional effect of incubating in helper factor. However, 10^6 lightly picrylated spleen cells caused only weak contact sensitivity and this was significantly increased by treatment with helper factor. The next three lines show various controls—the lack of effect of injecting normal spleen cells treated with helper factor or of injecting helper factor alone.

The following experiment investigated the antigen specificity of the T helper factor produced by supernatants of 4 day picryl or oxazolone immune cells using a criss-cross design. The top block of Fig. 2 shows that

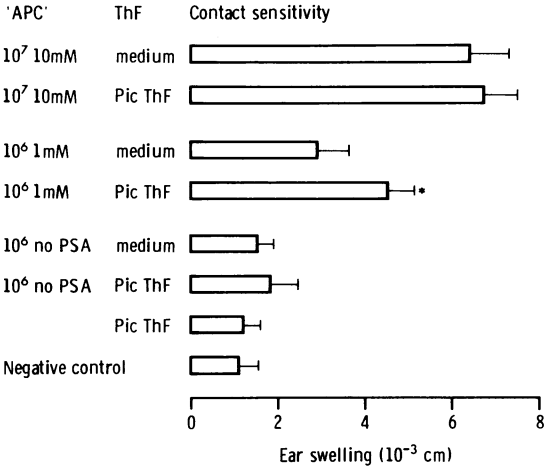

Figure 1. T helper factor augments contact sensitivity induced by suboptimal immunization. Ten or one million spleen 'antigen-presenting' cells ('APC') were picrylated with 10 or 1 mM picrylsulphonic acid (PSA) or left untreated. They were then treated with medium or T helper factor of picryl specificity (Pic ThF) and injected into the footpads of groups of five recipients and contact sensitivity assessed 5 days later. The bars show the increment of ear thickness at 24 hr measured in units of 10^{-3} cm $\pm$ standard deviation.

* Significantly different from 'APC' treated with medium: $P < 0{\cdot}02$.

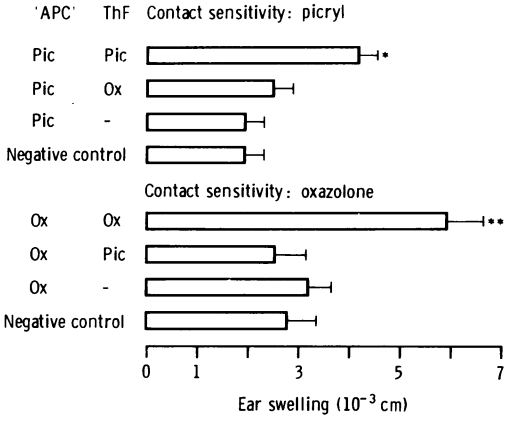

Figure 2. Antigen specificity of T helper factor. Spleen cells ('APC') were haptenized with picrylsulphonic acid or oxazolone and treated with picryl or oxazolone ThF or medium. They were then injected into the footpads of groups of five recipients and contact sensitivity assessed 5 days later. The top block shows contact sensitivity to picryl and the lower block to oxazolone.

Significantly different from mice given irrelevant T helper factor: * $P < 0{\cdot}02$, ** $P < 0{\cdot}001$.

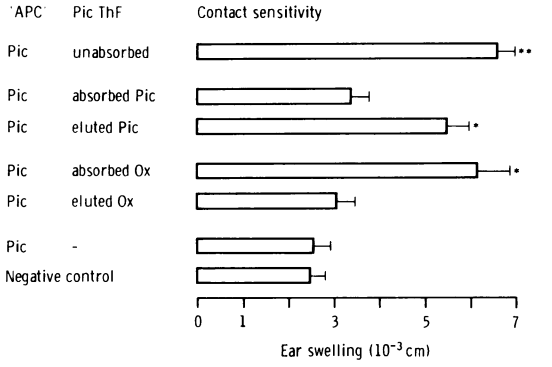

Figure 3. Specific absorption and elution of picryl T helper factor. Picrylated spleen cells ('APC') were treated with picryl T helper factor either unabsorbed or absorbed with or eluted from picryl or oxazolone microcrystalline cellulose.

lightly picrylated spleen cells incubated in picryl ThF caused significant contact sensitivity, while the same cells incubated in oxazolone ThF or in medium had no effect. The lower block shows similar results using oxazolonated spleen cells. It was concluded that the helper factor was antigen-specific.

The following experiment confirmed the specificity of ThF immunochemically. Partially purified ThF was prepared by affinity chromatography of the picryl ThF supernatant on picryl immunoabsorbent, while affinity chromotography on oxazolone immunoabsorbent provided control material. Figure 3 shows that picryl ThF could be purified with picryl but not with oxazolone immunosorbent. Similar results were obtained by absorbing picryl ThF supernatant with picrylated or oxazolonated spleen cells and the genotype of the absorbing cell (CBA or BALB/c) was unimportant (data not shown).

T helper factor is made by an Lyt-1^+2^-, I-A^+, I-J^-, Thy-1.2^+ T cell

Four day picryl immune cells were purified by nylon wool filtration and treated with various monoclonal sera or conventional anti-I-J antibody. They were separated by panning on anti-immunoglobulin plates—made with $F(ab')_2$ antibody to prevent the adherence of cells with Fc receptors—into marker positive and negative cells. These were then cultured at 10^7 ml^{-1} and the supernatant tested for ThF activity. Figure 4 shows that the ThF is made by an Thy-1.2^+ T cell with the usual phenotype of a helper cell—

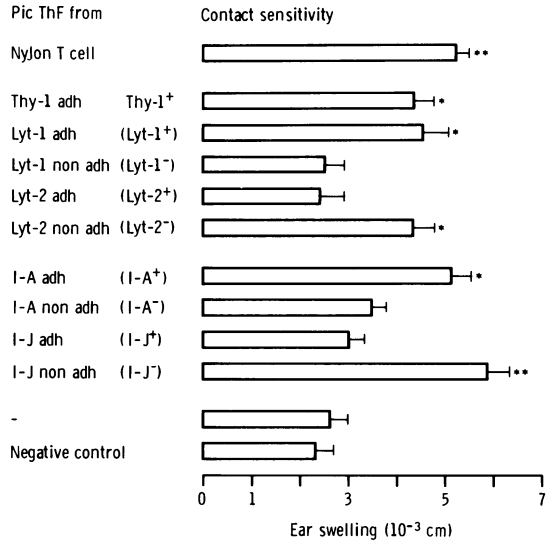

Figure 4. The production of ThF is dependent on a Thy-1$^+$ Lyt-1$^+$2$^-$, I-A$^+$, I-J$^-$ T cell. Four day nylon T picryl immune cells were treated with various monoclonal antibodies or conventional anti-I-J antibody. They were separated on anti-immunoglobulin plates into adherent and non-adherent fractions. The phenotype of the population is shown in parentheses. The cells were then cultured to produce ThF and tested on picrylated cells. Statistical significance is in comparison with the group which received no T helper factor. See legend to Fig. 2.

Lyt-1$^+$2$^-$, I-A$^+$, I-J$^-$. The I-A positivity of the cell was confirmed by abolishing its activity with monoclonal anti-I-A antibody and complement.

Dose–response and kinetics of production of T helper factor

In the following experiment mice were painted with various doses of picryl chloride. ThF was prepared from the lymph nodes 4 days later and tested for activity. The left hand panel of Fig. 5 shows that painting with 5% and to a lesser extent with 0·5% picryl chloride stimulated the production of ThF, while 0·05 and 0·005% picryl chloride was ineffective.

The right hand panel shows the kinetics of production. ThF was provided by lymph node cells taken 4 or 5 but not 13 days after painting. In another experiment (data not shown) 3 day and 4 day lymph node cells produced comparable amounts of ThF.

Molecular characteristics of T helper factor

Molecular weight. Picryl ThF was fractionated on Sephadex G-100 and pooled in bands of 10,000. Figure 6 shows that ThF has a molecular weight around 50,000–60,000.

T helper factor is I-A-positive. The following experiment investigated whether picryl ThF could be absorbed by monoclonal insolubilized anti-I-A of the appropriate (I-A^k) or inappropriate (I-A^b) specificity. Figure 7 provides the evidence that ThF bears I-A determinants.

I-A determinants and the antigen binding site may occur on a single molecular complex of ThF. It has already been shown that ThF binds to antigen and possess I-A determinants and can be recovered from

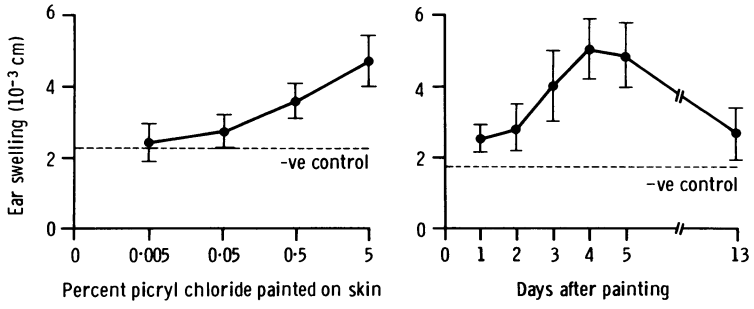

Figure 5. Dose–response and kinetics of the production of T helper factor. In the experiment shown in the *left hand panel*, mice were painted with various quantities of picryl chloride. The lymph node cells were taken 4 days later and their supernatant assayed for ThF activity. The negative control refers to mice which were left uninjected. The *right hand panel* shows the production of ThF by cells taken at various times after painting with 5% picryl chloride. The vertical bars show one standard deviation above and below the mean.

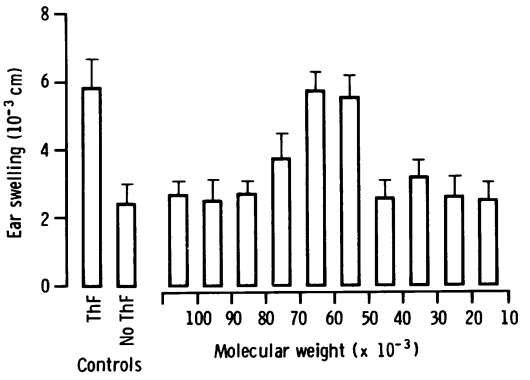

Figure 6. Molecular weight of T helper factor. ThF was fractionated on Sephadex G-100, the fractions pooled in bands of 10,000 and tested for helper activity. The two left hand columns refer to mice which received picrylated spleen cells incubated in the initial ThF or in medium only. Significant help ($P < 0{\cdot}02$) was only seen in the pools of 50,000–60,000 and 60,000–70,000.

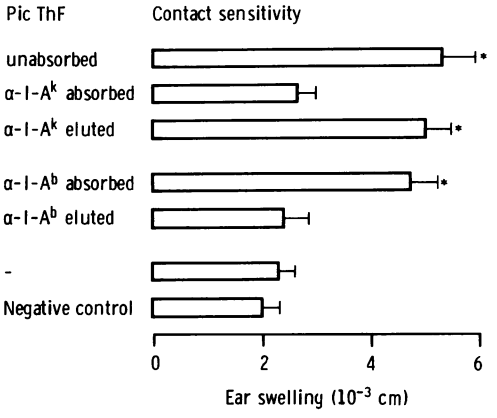

Figure 7. T helper factor bears I-A determinants. Picryl T helper factor of CBA (H-2^k) origin was tested unabsorbed or after absorption with or elution from insolubilized monoclonal anti-I-A^k (relevant) or anti-I-A^b (control) antibody and then tested for activity. Significance is in comparison to mice which received picrylated cells incubated in medium only. See legend to Fig. 2.

eluates of appropriate columns. To confirm further that the antigen binding site and the I-A determinants can occur on the same molecular complex, ThF was sequentially purified by affinity chromatography on antigen and anti-I-A antibody or visa versa. Figure 8 shows that this double purified ThF retained biological activity.

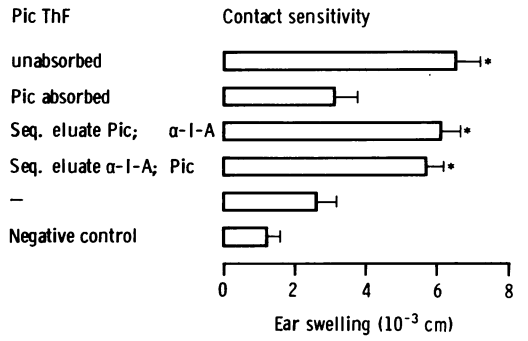

Figure 8. T helper factor both binds to antigen and bear I-A determinants. Picryl T helper factor was sequentially absorbed with and eluted from picryl beads followed and anti-I-A or visa versa and then tested for activity. See legend to Fig. 2.

DISCUSSION

These results bring contact sensitivity in line with other responses, such as antibody production (Nakajima *et al.*, 1983; Lonai, Puri & Hammerling, 1982; Mozes, Eshhar & Apte, 1982; Taussig, 1974; Feldmann & Basten, 1972) and T cell cytotoxicity (Kilburn *et al.*, 1979; Plate *et al.*, 1982) in which antigen-specific T helper and augmenting factors occur. The present T helper factor shows antigen specificity both biologically and immunochemically. It occurs as a single molecular complex and, like other antigen specific helper factors, has, a molecular weight around 60,000 and bears I-A determinants. Moreover, it is produced by a cell with the Lyt-1^+2^-, I-A^+, I-J^- phenotype which is characteristic of helper cells (Tada & Okumura, 1979; Altman & Katz, 1980; Taussig, 1980).

The helper factor for the induction of contact sensitivity is similar or identical to the antigen binding T cell factor which augments the effector stage of the contact sensitivity reaction (Askenase, Rosenstein & Ptak, 1983; Ptak *et al.*, 1982). Its ability to provide help for antibody production was not studied. However, both the T helper cells for antibody production and the T cell which make the helper factor for contact sensitivity, occur in the regional lymph nodes but not in the spleen of mice painted with contact sensitizer (Thomas *et al.*, 1978).

How does T helper factor act? A simple view is that it attaches to haptenized cells by its antigen binding site and augments the immune response by virtue of its I-A determinants. In other words, it associates antigen with I-A. An alternative view is that it is cytophilic for

antigen- presenting cells, which then release non-specific mediators such as interleukin-1 when exposed to antigen and major histocompatibility complex products (MHC). Its biological function may be to provide an amplification loop which comes into action about the time that T helper factor first appears, i.e. day 3 after immunization.

However, T helper factor may play a more important role at the beginning of the immune response. The idea that antigen-specific factors may be essential for the initiation of the immune response has a long history. Jerne in his natural selection theory of antibody production suggested that the combination of antigen with antibody was an essential early step in the immune response, and the possible importance of antibody cytophilic for macrophages was discussed by Nelson & Boyden (1967). Lachmann (1971) proposed that a T cell product (antigen-dependent migration inhibition factor) was cytophilic for macrophages and was required for antigen presentation, while Feldmann (1972) and Feldmann & Basten (1972) showed that T helper factor acted through adherent cells, and suggested that factor–antigen complexes adhered to the antigen-presenting cells and initiated the immune response. Raff (1982) discussed whether the antigen-presenting cell requires a special cue to present antigen, i.e. to associate antigen with I-A on its surface and to produce interleukin 1. In some cases bacterial products such as endotoxin and cell wall adjuvants may provide the cue but it is not obvious how bland antigens activate the antigen-presenting cell. One possibility is that there is a spontaneous background level of production of antigen specific T helper factors even in unimmunized animals, that these natural factors are cytophilic for antigen-presenting cells and that exposure of the armed antigen-presenting cell to antigen together with MHC activates the cell and initiates the immune response perhaps by the production of interleukin-1.

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