

# The Cellular Infiltrate of the Contact Sensitivity Reaction to Picryl Chloride in the Mouse

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Contact sensitivity to picryl chloride was studied in the mouse ear. The skin hypersensitivity, as a factor of ear swelling, and the intensity of the cellular infiltrate in the skin were evaluated together during the elicitation of the contact sensitivity reaction. It was found that the ear swelling is a mainly vascular reaction preceding the appearance of inflammatory cells. The infiltrating cells in this model of skin sensitivity were differentiated in Giemsa-stained plastic-embedded sections and recorded in a semi-quantitative way. When the cellular reaction was most intense at 48 and 72 hours after challenge, the most numerous inflammatory cells were lymphocytes and eosinophils. *Key words: Contact sensitivity; Picryl chloride; Mouse; Ear swelling; Histology; Cell differentiation.* (Received October 6, 1982.)

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Contact sensitivity in the mouse can be quantified in various ways: from inspection of the test reaction with the naked eye, or skin thickness measurement with callipers (1, 4), to more elaborate methods which estimate the accumulation of radioactively labelled inflammatory cells at the test site (3, 12). The various methods concentrate on different aspects of the inflammation evoked by the sensitizing agent, i.e. the edema and the cellular infiltrate respectively.

The histopathological features of the contact allergic reaction in guinea pigs and humans have been studied in detail, but the information on the contact sensitivity reaction in the mouse is less explicit (5, 9). The present investigation deals with the quantitative evaluation of the cellular infiltrate of the contact hypersensitivity reaction in the mouse.

## MATERIALS AND METHODS

### *Animals*

The animals used were inbred, male CBA-mice, 8-10 weeks old, obtained from Anticimex, Stockholm, Sweden. They were caged in groups of 5 and fed on standard mouse pellets and water *ad libitum*.

### *Sensitization*

The animals were sensitized with 0.2 ml of 7% picryl chloride (PiCl) in methyl-ethyl-ketone (MEK) as described previously (3). Non-sensitized control animals were given MEK only.

### *Challenge*

Three days after sensitization or sham treatment the mice were challenged with 20  $\mu$ l of 0.5% PiCl in MEK on both sides of the right ear and with MEK only on both sides of the left ear. The procedure was carried out under light ether anaesthesia.

### *Ear swelling test*

Contact sensitivity was assessed at 6, 12, 24, 48 and 72 hours after challenge. At these intervals, groups of mice were killed by cervical dislocation and the thickness of the ears measured with an

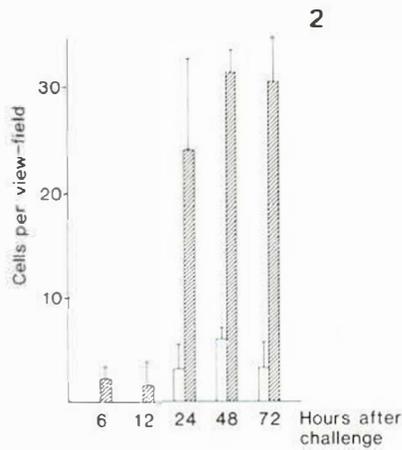
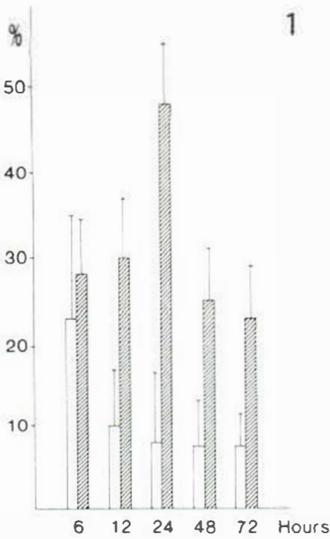


Fig. 1. The ear swelling, expressed as a percentage, at various times after antigen challenge in non-sensitized (open bars) and sensitized (hatched bars) mice (mean  $\pm$  SD).

Fig. 2. The net cellular response, as defined in Materials and Methods, of all cells together in test reactions from non-sensitized (open bars) and sensitized (hatched bars) mice at different times after antigen challenge (mean  $\pm$  SD).

engineer's micrometer. The ear swelling was expressed as a percentage according to the following formula:

$$\frac{\text{thickness of (test ear - control ear)}}{\text{thickness of control ear}} \times 100$$

#### Histological technique

The ears were fixed in cold neutral-buffered formalin and, after dehydration in alcohol series, embedded in glycol methacrylate and polyethyleneglycol (JB-4, Plastic Embedding Kit®, Polysciences, Warrington, Pa., USA). Sections, 3  $\mu$ m thick, were stained with May-Grünwald-Giemsa.

The cells in corium between the epidermis and the ear cartilage were counted in 20 consecutive high-power fields (1250 $\times$ , oil immersion). The test and control ear from each mouse were both examined and the cells divided into lymphocytes, mast cells, eosinophils and neutrophils. The rest—consisting of fibroblasts, endothelial cells, monocytes, macrophages, occasional basophils, and unidentified cells—were classified as mesenchymal cells. The net cellular response was calculated as the number of differentiated cells per field of test ear, minus the number of cells per field of the control ear from the same mouse.

## RESULTS

The degree of ear swelling at various times after challenge is shown in Fig. 1. It is evident that there was an initial non-specific swelling 6 hours after challenge in both sensitized and non-sensitized animals. This non-specific reaction decreased considerably during the next 6-hour period. The specific hypersensitivity showed a peak at 24 hours and then levelled off to a plateau for the period up to 72 hours.

The calculated net cellular response of all cells together is presented for both sensitized and non-sensitized mice in Fig. 2. The accumulation of inflammatory cells at the test site had a time-schedule that differed from that of the ear swelling. The differential counts of various cell types in the control ears of all animal groups were fairly constant, which vouches for the reliability of the cell differentiation. The cellular response in non-sensitized animals constitutes a toxic reaction to the PiCl application. Thus the difference between the cellular responses of sensitized and non-sensitized animals may be considered a true contact allergic cell infiltrate.

The cellular infiltrate at various intervals after challenge is subject to some variation. At

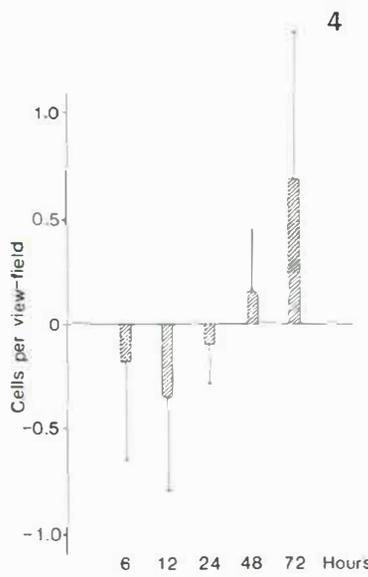
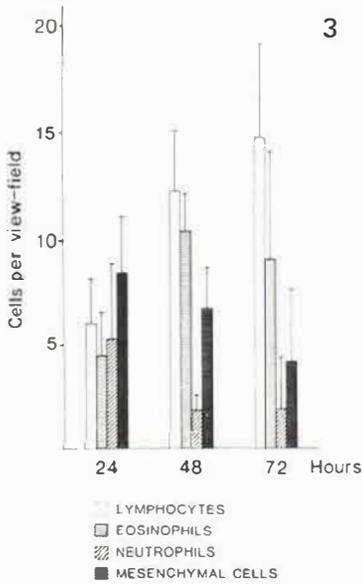


Fig. 3. The differential counts of the net cellular response, as defined in Materials and Methods, in test reactions from sensitized mice (mean  $\pm$  SD).

Fig. 4. The net cellular response (mean  $\pm$  SD) of the mast cells in the test reactions from sensitized mice. The difference between 12-hour and 72-hour values is significant ( $p < 0.05$ ).

24 hours after antigen application, when the ear swelling was most prominent, lymphocytes, eosinophils, neutrophils and mesenchymal cells constituted equal parts of the cell population. Later, in the 48- and 72-hour reactions, lymphocytes and eosinophils predominated (Fig. 3).

The mast cells comprised about one-third of the total cell population in the control ears. In the contact sensitivity reaction the absolute number of mast cells did not show any considerable variation, but the calculated net cellular response demonstrated a biphasic development with negative values early in the hypersensitivity reaction, followed by positive values later on (Fig. 4). The difference between the 12-hour and 72-hour values is significant ( $p < 0.05$ ).

## DISCUSSION

In the present investigation it was clearly shown that the antigen-induced ear swelling is a mainly vascular reaction, since the ear swelling at 12 and 72 hours was almost the same, while the cellular infiltration had not started at 12 hours but was heavy at 72 hours. These findings are in agreement with those of Möller (8) who showed a net weight increase of the test ear, reaching a maximum at 24 hours. Thus there seems to be a dissociation between the vascular reaction and the intensity of the cellular infiltrate with respect to time.

The cellular infiltrate increased significantly between 12 and 24 hours and the influx of cells consisted of lymphocytes, eosinophils, neutrophils and so-called mesenchymal cells. After 24 hours the lymphocytes and eosinophils showed a further increase, while neutrophils and mesenchymal cells decreased in number. The mast cells showed a negative net cellular response in sensitized animals during the first 24 hours after challenge. Whether this is due to degranulation or migration could not be determined in this study. However, it has been demonstrated that vasoactive amines participate in the delayed hypersensitivity response in the mouse (6), and that antigen-specific degranulation of the mast cells will occur in picryl chloride sensitive animals in the early phase of the reaction (2). This degranulation can be found on day 4 after sensitization, but reaches a maximum later (10).

Thus the decrease in number of mast cells may be due to degranulation. The mast cells specifically are important because the mouse virtually lacks basophils, which are abundant in delayed-type skin reactions in other species. Recently it has been reported that basophils may be mobilized in the mouse, at least in some strains (11). The cells, however, have a rather different appearance and may be overlooked in histological sections stained with Giemsa. Further electron microscopical studies will probably clarify this issue.

The considerable accumulation of eosinophils at the test site is also of interest. Tissue eosinophilia may be the result of chemotaxis or specific eosinophil trapping. Both these mechanisms may operate through the pharmacological effects of histamine (13). Thus the increase in eosinophils in the test reaction may be secondary to the mast cell release of histamine.

Previous studies have demonstrated that cell populations in delayed skin hypersensitivity may vary when the test reactions are elicited sooner or later after sensitization (7). This fact must be borne in mind in the interpretation of the kinetics of the cell population of the cellular infiltrate in the present investigation.

The quantitative measurement of the cells accumulated in the contact sensitivity skin reaction provides information which may be useful for further evaluation of the delayed skin reaction and its modulation by various pharmacological agents.

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