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THE CELLULAR INFILTRATE IN CONTACT HYPERSENSITIVITY TO PICRYL CHLORIDE IN THE MOUSE

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Abstract. In the present work, the contact hypersensitivity skin test reaction to picryl chloride in CBA mice was examined. The test was performed by applying the contact allergen to the ear skin and making a series of histological analyses up to 24 hours after challenge. An increment in ear thickness, measured with an engineer's micrometer 24 hours after challenge, was obvious in a group of sensitized mice when compared with a non-sensitized control group, and the difference was found to be highly significant. One hour after challenge, mononuclear cells appeared in the dermis, increasing in numbers during the following 12 hours. At this time, neutrophil granulocytes were the dominant cells in the infiltrate and remained so up to 24 hours after challenge. On the basis of the experiments performed here we conclude that measuring of the ear swelling with a micrometer 24 hours after challenge is a useful and reliable test of contact sensitivity in the mouse.

In studies on allergic contact dermatitis, the guinea pig is frequently used as an experimental animal. Two extensive monographs on eczema in guinea pigs have been published during recent years (7, 8). Contact hypersensitivity can also be induced in mice and successful sensitization experiments have been performed in the CBA mouse with picryl chloride, 2-phenyl-4-ethoxy-methylene oxazolone and dinitrochlorobenzene (1). In these experiments, ear challenge has been performed to study inflammation (1), instead of testing on mouse flank skin, which becomes easily irritated by shaving.

This study describes the histological findings after application of picryl chloride (PiCl) to the ear of previously sensitized and non-sensitized mice.

MATERIAL AND METHODS

Animals

Inbred, male, 3-month-old CBA/Lü mice, fed on pellet diet and water ad libitum, were used. The animals were bred at the Karolinska Institute, Stockholm, Sweden.

Sensitization, challenge and quantification of contact hypersensitivity were performed as described previously (1, 13, 15). Mice were sensitized epicutaneously with 0.1 ml of 7% PiCl (British Drug Houses Ltd, Poole) diluted in absolute alcohol and applied to the clipped abdomen. Epicutaneous challenge was performed with PiCl 1% dissolved in olive oil, 0.05 ml was applied to both sides of the ear and 24 hours after challenge the increment in ear thickness was measured with an engineer's micrometer, 8 mm in diameter. Mean increment in ear thickness of the two ears of each mouse was calculated and the result expressed in units of 10^{-2} mm. The value for each group of mice was expressed as mean ± standard error of the mean. Student's *t*-test was used in the statistical comparison of different groups. The numbers of mice in the various groups are to be found in Table I.

Twenty-four mice were placed simultaneously in a cage. They were sensitized and challenged according to the above-mentioned procedure. After challenge, the right ear was taken for histological examination after 1, 3, 6, 9, 12 and 24 hours, four mice at a time.

Histological methods. The ears were removed at the base, divided into two parts, one of which was fixed in 10% neutral formalin and embedded in Sorvall JB-4 methacrylate. A transverse section 2 μm thick was cut and stained by the May-Grünwald-Giemsa method.

In the sections, the tissue distribution of the inflammatory changes was registered and an analysis of the inflammatory cells was made. The methacrylate embedding and Giemsa staining permit differentiation between various types of granulocytes. The cells registered as mononuclear were those having a well-defined cytoplasm, a single nucleus, and a morphology similar to mononuclear cells, as found in blood or lymph vessels (Fig. 1). Thus cells considered as lymphocytes had a round nucleus with a sparse or moderate amount of cytoplasm. Distinction between macrophages and monocytoid cells could not be performed in all instances and cells registered as belonging to the macrophage-monocytoid group were larger than the lymphocytes and had a folded nucleus, often with an uneven nuclear margin and a more abundant cytoplasm.

Method error was determined by two consecutive registrations of mean increment in ear thickness in 20 non-sensitized and non-challenged animals. The registrations were made by one person. The following formula was used: $V[\Sigma(d_i)^2/2n]$ (5). The method error was found to be 0.8.

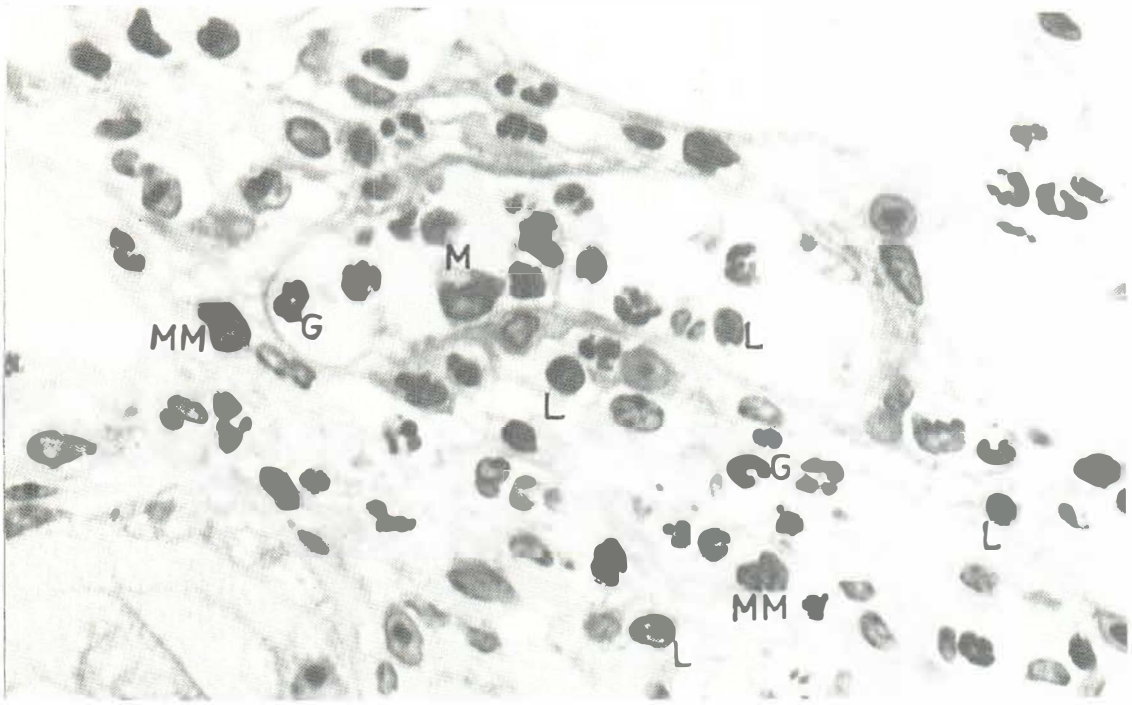

Fig. 1. Examples of intra- and extravascular cells at 12 hours. The upper part shows a blood vessel with monocytes (*M*), granulocytes (*G*) and lymphocytes (*L*). Below the vessel are extravascular inflammatory cells in the dermis, cells of monocyte-macrophage character (*MM*), granulocytes (*G*), and lymphocytes (*L*). Giemsa, ×650.

RESULTS

In Table I, comparison can be made between the intensity of the contact skin reaction induced 6 days after epicutaneous sensitization of CBA/Lü mice with PiCl 7% (group 1) and the intensity of the reaction in three groups (4, 5 and 6) where no sensitizing substance was applied. Challenge was performed with PiCl 1% in all experiments except in groups 5 and 6, where the effect of the vehicle (olive oil) and the pressure effect of the micrometer were studied. The effect of sensitization is clear. Ear thickness in PiCl-sensitive mice 24 hours after challenge was found to be significantly greater in the sensitized group than in non-sensitized animals on challenge with PiCl 1% ($p<0.001$), olive oil ($p<0.001$) and after pressure applied to the ear by the micrometer ($p<0.001$). These results indidate an underlying immunological response in group 1.

Histologically, inflammation was found in the ears of the sensitized mice. The inflammation was located in the dermis and was most pronounced in the lower half of the ear. After 6 hours a slight oedema in the dermis was observed. This oedema

Table I. *Increment in ear thickness of the various groups of mice*

Group	Sensitization	Challenge	Increment in ear thickness 10^{-2} mm	No. of mice
1	PiCl 7%	PiCl 1%	5.3±0.6	10
2	0	PiCl 1%	0.9±0.4	18
3	0	Olive oil	0.9±0.5	8
4	0	0	1.2±0.4	9

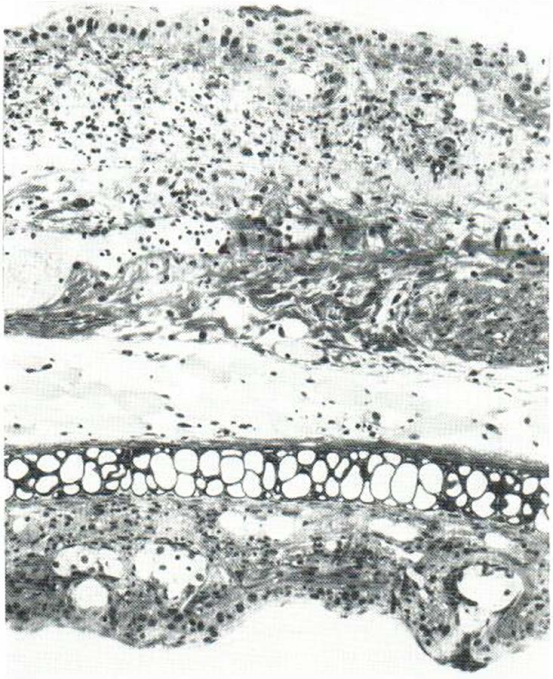

Fig. 2. Section through ear of sensitized mouse 24 hours after ear challenge with 1% picryl chloride, showing dermal oedema with a rich cellular infiltration. Giemsa, ×100.

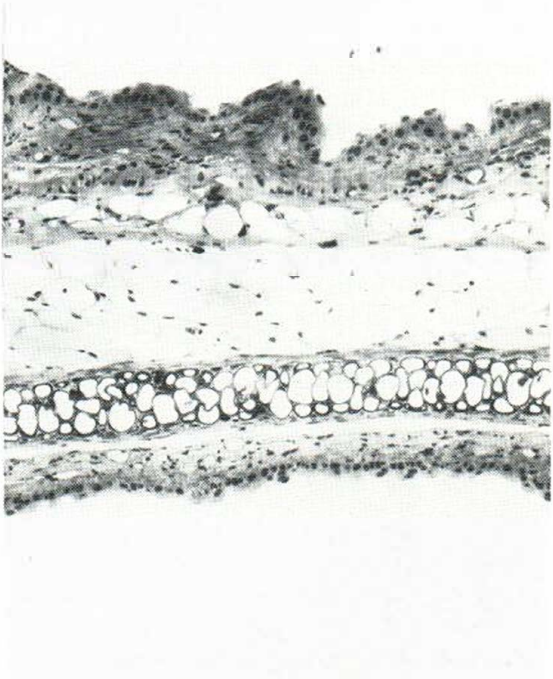

Fig. 3. Section through ear of non-sensitized mouse 24 hours after ear-painting with 1% picryl chloride. The dermal structure is preserved. Occasional granulocytes are seen in the dermis. Giemsa, ×100.

was progressive and 12 hours after challenge it was accompanied by disruption of the collagen stroma in the areas where inflammation occurred.

The cells registered as mononuclear appeared in the sections 1 hour after challenge. Up till 9 hours after challenge the dermal mononuclear cells were predominantly monocytoid and macrophages, although occasional lymphocytes were found. At 9 and 12 hours, when the inflammation was more pronounced, the large mononuclear cells of monocytoid-macrophage character still dominated among the mononuclear cells, although cells with lymphocyte morphology seemed more abundant than in the earlier phases.

During the first 3 hours no increase in eosinophils or neutrophils occurred, but after 6 hours there was an accumulation of neutrophils which increased gradually throughout the observation period. The eosinophils started accumulating at 9 hours and increased slightly in number up to 24 hours after challenge.

The ear swelling, 24 hours after challenge (Fig. 2), was mainly caused by oedema and disruption of the collagen stroma. The cellular infiltrate was composed chiefly of neutrophils, but also exhibited increase numbers of eosinophils. Basophilic granulocytes and mononuclear cells were occasionally found. The numbers of mast cells in the ears of sensitized animals had decreased in the areas of inflammation, compared with those in normal ears. In the epithelium a focal spongiosis together with a few granulocytes was observed after 9 and 12 hours. At 24 hours there were in som ears small superficial erosions with infiltration of granulocytes.

In the ears of the non-sensitized animals 24 hours after painting, no disruption of collagen stroma or oedema could be observed by light microscopy. A

slight infiltration of neutrophils was seen (Fig. 3) but no increase in eosinophils or mononuclear cells could be found.

DISCUSSION

The observations presented here show that application of PiCl 7% to the abdomen of mice 6 days before challenge with PiCl 1% produces a significant increase in ear thickness, compared with groups of mice which have only received a challenge dose of PiCl 1% in olive oil or which have been exposed to the pressure effect of the micrometer. Histologically, inflammation was also considerably more pronounced in the ears of the sensitized animals than of the control mice. A mixed reaction with humoral and cellular components has been suggested in studies concerning contact sensitivity to oxazolone in the mouse (2). The finding of polymorphonuclear leukocytes in the lesions (16), the ability to transfer the skin reaction by immune serum (18), and a swelling of the ear as early as 4 hours after challenge (1) have indicated an antibody-mediated oxazolone reaction, while the ability to transfer the reaction with lymphocytes implies a delayed hypersensitivity component (2).

Transfer of contact sensitivity to PiCl by lymph node cells in the mouse has been successful (3), while transfer with serum has failed (18).

In the guinea pig the mononuclear cell dominates the infiltrate in intense contact hypersensitivity reactions (8). In the mouse, however, the polymorphonuclear cell response is found to be more vigorous than in the guinea pig (4) and our finding of a contact hypersensitivity reaction starting with mononuclear cells and followed 24 hours after challenge by an infiltrate with polymorphonuclear cells, is in agreement with the results of other investigators using oxazolone (1, 6, 10, 16), dinitrofluorobenzene (DNFB) (11) and PiCl (1) as allergen and using the ear challenge technique.

The possible tissue origin and the functional state of the mononuclear cells observed in the present experiment cannot be predicted by light microscopic observation alone. However, recent results indicate that lymphocytes *in vitro* may release substances that are chemotactic for granulocytes (12). Mononuclear cells appeared prior to the granulocyte accumulation and the possibility of a similar mechanism working *in vivo*, as an explanation of the composition of the cellular infiltrate 24 hours after challenge, must be considered.

Where mast cells are degranulated, eosinophils appear to phagocytize the mast cell granules (9). In the present study an increase in eosinophils could be found in the ears of sensitized mice 24 hours after challenge and also a reduced number of mast cells. This finding also supports the assumption that mast cells may play a part in this reaction too. In fact, using the same experimental model the synthesis of a reaginic antibody within 7 days of epicutaneous application of PiCl has been described (17) and increased amounts of urine histamine have been found 24 hours after challenge (14).

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