Histological and Immunopathological Studies of Delayed Hypersensitivity Reaction to Tuberculin in Mice

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At 4 to 6 weeks after intravenous infection with 2 × 10⁶ CFU of dispersed Mycobacterium bovis bacilli (BCG), C3H/HeNCrIBR and C57BL/6NCrIBR mice exhibited a strong reaction to purified protein derivatives, as evaluated by the increase in footpad swelling at both 24 and 48 h after local antigenic challenge. However, histological studies of the footpad skin demonstrated a prominent perivascular infiltration with polymorphonuclear cells at 6 and 24 h after purified protein derivative challenge, whereas mononuclear cells represented the majority of infiltrating cells only at 48 h. An immunopathological study of the footpad skin showed granular deposits of immunoglobulins and complement in vascular walls and perivascular tissues at 6 and 24 h. These results demonstrate that the footpad swelling observed 24 h after the antigenic challenge is caused by an Arthus-type reaction, whereas that caused by cell-mediated immunity appears at 48 h. Hence, delayed hypersensitivity must be evaluated at 48 and not 24 h after challenge.

The development of delayed-type hypersensitivity to microbial antigens is a frequent consequence of infection with facultative intracellular parasites (4, 6). A number of methods evaluating a delayed hypersensitivity reaction (DHR) have been described. Intradermal challenge with purified protein derivatives (PPD) or tuberculin in Mycobacterium bovis BCG-infected rats and guinea pigs are able to elicit a good DHR (2, 9). Although Crowle (5) was able to detect tuberculin hypersensitivity of BCG-infected mice by intradermal PPD testing, it is generically believed that footpad swelling upon tuberculin challenge is a better measurement of DHR in this particular species (7).

Traditionally, this reaction is described as a slowly evolving inflammatory lesion which reaches a maximum at ca. 24 h after the challenge and is still present after 48 h (4). Histological studies of the cutaneous lesion in guinea pigs demonstrated a predominantly mononuclear cell infiltrate, without necrosis, at 24 h postchallenge (10). In mice, however, the lesion was characterized by an infiltrate that was predominantly neutrophilic (1, 4). The possibility that these early lesions in mice represented a mixture of the Arthus reaction and DHR was suggested but not convincingly proven, since immunopathological studies were not done (1, 15). This communication provides evidence that the Arthus reaction is, indeed, responsible for the early (24-h) footpad swelling in BCG-infected mice challenged with PPD. The evidence is based on both the histological appearance as well as on the presence of immune reactants at the sites of antigen injection.

Male C3H/HeNCrIBR (C3H/HeN) and C57BL/6NCrIBR (C57BL/6N) mice, weighing 20 g, were used for the experiments. Mice were infected intravenously with 0.25 ml of BCG suspension containing 2 × 10⁶ CFU, by a method already described (8). Previous results from our laboratories demonstrated that mice infected according to this protocol developed the highest degree of PPD-induced DHR at 4 to 6 weeks after infection (13). At these time periods, infected mice were challenged by 5 μg of PPD (Statens Serum Institut, Copenhagen, Denmark) in 0.025 ml of saline into the right hind footpad, and the increase in footpad swelling was compared with that of the contralateral footpad injected with saline alone at 6, 24, and 48 h after challenge as measured by dial gauge calipers. Uninfected mice were challenged with PPD to serve as controls. After mice were killed by exsanguination, footpad skin, including subcutaneous tissues all the way to metacarpal bones, was removed and fixed for histological examination or frozen for immunopathological studies. Frozen sections were incubated with fluorescein-conjugated goat anti-mouse immunoglobulin G (IgG). IgM (Meloy Laboratories Inc., Springfield, Va.), and C3 antiserum (Cappel Laboratories Inc., Cochranville, Pa.). All the antisera have been tested on positive kidneys from NZB mice with glomerulonephritis.

When evaluated by the increase in footpad swelling, the DHR was significantly different (P < 0.001) at both 24 and 48 h after the PPD challenge in BCG-infected C3H/HeN and C57BL/6 mice when compared with uninfected controls, although the highest reactivity was observed at 48 h (Fig. 1). Histological study of the footpad skin showed tissular edema and a prominent perivascular and tissular infiltration mostly by polymorphonuclear cells at 6 and 24 h (Fig. 1). In addition, vasculitis was present at these time intervals. Necrotic vascular walls were replaced by fibrinoid material and infiltrated by polymorphonuclear cells (Fig. 2a). Histological study of the footpad skin from control noninfected mice did not demonstrate any obvious cellular infiltration. Immunopathological study showed granular deposits of IgM and C3 in vascular walls and perivascular tissue in both strains of mice 6 and 24 h after injection of PPD (Fig. 2b). Histology of footpad skin at 48 h after PPD challenge revealed tissular dissociation by edema and infiltration mostly by mononuclear cells with only occasional polymorphonuclear cells. Deposits of IgM and C3 were considerably less prominent than at 24 h. Tissues from control, noninfected mice injected with PPD and from tissues from the infected mouse contralateral footpad which was injected with saline alone were negative on immunofluorescent examination.

Footpad swelling is considered a relevant measure of the DHR of mice to various facultative intracellular parasites,
especially mycobacteria (6). This reaction has traditionally been described as an inflammatory event which reaches a peak at 24 to 48 h after antigenic challenge, and different investigators have measured the DHR interchangeably at either 24 or 48 h (3, 8, 11, 14). Other studies, however, noted that the largest footpad reactivity was obtained only at 48 h after antigenic challenge (12). Our results clearly show that the highest DHR, evaluated by the increase in footpad swelling, is observed at 48 h after challenge with PPD in BCG-infected animals and that mononuclear cells constitute the majority of the inflammatory cells only at 48 h. At earlier intervals, the lesions are characterized by a predominant polymorphonuclear cell infiltration, and fibrinoid necrosis of the vascular wall can be seen. Demonstration of IgM and C3 deposits in vascular walls and perivascular tissue after 6 and 24 h provides the evidence for an Arthus-type reaction. The absence of IgG is not explained at the moment, but it should be noted that the presence of IgM antibodies as the sole molecular species of immunoglobulins is often evident in many immune complex diseases, both in mice and in humans. Although IgM and C3 deposits had persisted in tissues for up to 48 h, they were much less prominent at that time than at 6 and 24 h. Persistence of these deposits after 48 h can be explained by incomplete denaturation of immune complexes.

DHR is a lymphokine-mediated reaction that develops in a series of steps (4). After the injection of the antigen, there is an influx of sensitized T lymphocytes that interact with the antigen locally. Upon stimulation by the antigen, lymphocytes release soluble factors, the lymphokines, some of which will attract and activate other lymphocytes and macrophages. Lymphokines and macrophage products produce an inflammatory reaction characterized by erythema, edema, and cellular infiltration. Considering these immunopathological mechanisms, it appears that the measure of DHR by the increase in footpad swelling is detecting the presence or absence of edema. We propose that histological study of the
footpad skin is a method of measuring DHR which is reliable and accurate and which allows a qualitative and semiquanti-
tative determination of inflammatory cellular infiltration.
Moreover, there was a good correlation between footpad swelling and mononuclear cell infiltration at 48 h after PPD
challenge.

In conclusion, our studies demonstrate that DHR, when
evaluated by the increased footpad swelling, should be
measured at 48 h after antigenic challenge in BCG-infected mice. The increase in footpad swelling that occurs at 24 h
after the challenge is secondary to an Arthus-type reaction.
Also, it appears that the histological determination of in-
flammatory cellular infiltration is an accurate measurement of
DHR.

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