Clin. exp. Immunol. (1988) 71, 428–432

Enhancement of DTH reaction and inhibition of the expression of class II transplantation antigens by *in vivo* treatment with antibodies against γ-interferon

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(Accepted for publication 7 October 1987)

SUMMARY

During the delayed type of hypersensitivity (DTH) reaction in the skin, class II transplantation antigens are expressed on the keratinocytes. This induction is attributed to the action of γ-interferon (IFN-γ). We have now studied the influence of antibodies against IFN-γ on the DTH-reaction. Lewis rats were sensitized to 2,4-dinitro-1-fluorobenzene (DNFB) and challenged 5 days later with DNFB on the ears. Immediately before challenge each animal in one group ($n = 16$) was given 1 mg of mouse monoclonal antibodies (MoAb) against rats IFN-γ, denoted DB-1, intraperitoneally (i.p.), another group ($n = 15$), 1 mg of an irrelevant MoAb and a third group ($n = 11$) was left untreated. The ear thickness was measured with a micrometer, before challenge and after 24, 48 and 72 h. At 72 h all rats were killed, the ears cut off, snap frozen and stained with immunoperoxidase using MoAbs OX 6 and OX 17, directed against rat class II antigens. The DB-1 treated group was found to have a larger ear swelling that was statistically significant at each time point compared with the other two groups. Furthermore, the animals given DB-1 showed class II antigen expression on Langerhans' cells, but almost none on keratinocytes. In contrast, the rats in the two other groups displayed a moderate to strong expression of class II antigens on keratinocytes as well as on Langerhans' cells. It is concluded that DB-1 can inhibit class II antigen expression on keratinocytes during the DTH- reaction and also enhance the local response.

Keywords DTH-reaction γ-interferon monoclonal antibodies class II antigens

INTRODUCTION

The development of a delayed type of hypersensitivity (DTH) reaction in the skin is characterized by an early oedematous phase followed by a later infiltration of inflammatory cells (Turk, 1980). The inflammatory reaction and the activation of macrophages is dependent on antigen-specific T-helper cells (Bianchi *et al.*, 1981) which secrete a number of lymphokines with regulatory and/or inflammatory promoting functions (Krammer *et al.*, 1983). One of these factors is gamma-interferon (IFN-γ), known to induce expression of class II transplantation antigens on a variety of cell types, including keratinocytes (Basham *et al.*, 1984; Scheynius, Johansson & van der Meide, 1986). The local secretion of IFN-γ by activated T-helper cells may thus mediate the observed induction of class II antigens on keratinocytes in the late phase of the DTH reaction (Scheynius & Tjernlund, 1984).

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We have previously suggested that the development of class II antigen expression on keratinocytes may contribute to a possible suppressive, rather than enhancing effect on the immune response (Scheynius & Tjernlund, 1984). In this study we have treated rats with mouse monoclonal antibodies (MoAbs) against IFN-γ to investigate its influence on the IFN-γ mediated induction of class II antigens on keratinocytes and the local response during the DTH reaction.

MATERIALS AND METHODS

Animals

Lewis rats of both sexes, originally obtained from Benton & Kingham (UK), were bred in our own breeding facilities and used at 2- to 4-months of age. They were fed with pellets and allowed free access to water.

Sensitization and elicitation of contact sensitivity

Twenty μl of 0·5% 2,4-dinitro-1-fluorobenzene (DNFB) (Sigma Chemical Company, St Louis, Mo, USA) in 4:1 acetone:olive

oil (F. Merck, Darmstadt, W. Germany and Sigma Chemical Co.) was painted on the shaved abdominal wall skin on days 0 and 1. On day 5 the rats were challenged by applying 20 μl of 0·2 % DNFB, in the same vehicle, on the dorsal side of each ear. The ear thickness was measured before challenge and also after 24, 48 and 72 h, using an engineer's micrometer (Mitutoyo CO MFG Ltd., Tokyo, Japan). Each lobe was measured at three separate loci, yielding six observations per animal. The results are expressed as increase in ear thickness (mean value of both ears at each measurement minus mean value before challenge). Unsensitized animals painted with DNFB on the ears in the same way and normal rats served as controls. All experiments were performed under ether anaesthesia.

Production of mouse monoclonal antibodies

The IgG1 producing mouse hybridoma DB-1 is reactive with rat IFN-γ (van der Meide *et al.*, 1986). Another hybridoma, producing IgG2b antibodies with irrelevant specificity denoted G11 (Juhlin *et al.*, 1987) was used as a control. The hybridoma cells were cultured in Dulbecco's modified Eagles medium (Flow Lab, Irvine, UK) supplemented with 2 mM L-glutamine (Sigma), 50 IU penicillin and 60 μg streptomycin per ml, 3% fetal calf serum (KC Biological, Lenexa, Kansas, USA) and 5×10^{-5} M 2-mercaptoethanol. The supernatants were obtained by centrifugation and the pH adjusted to 8·1. The supernatants were thereafter applied on protein A Sepharose columns (Pharmacia, Uppsala, Sweden) and the antibodies were eluted at pH 5 (DB-1) and pH 3 (G11), respectively. The eluates were concentrated by ammonium sulphate precipitation, dialysed against phosphate buffered saline (PBS), sterile filtered and stored at −70°C. The antibody contents were quantified by spectrophotometric analysis at 280 nm. The isotypes of the antibodies were determined with ELISA using isotype specific peroxidase conjugates (Nordic, Tilburg, The Netherlands).

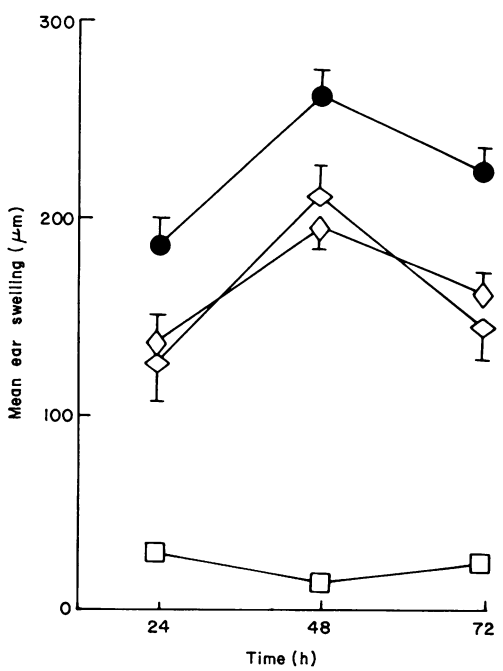

Fig. 1. Mean ear swelling ± s.e.m. after DNFB challenge in three groups of sensitized rats. Immediately before challenge the animals were given 1 mg DB-1, a MoAb against rat IFN-γ i.p. (●; $n=16$), 1 mg G11, an irrelevant MoAb (◇; $n=15$) or were left untreated (; $n=11$). Unsensitized but DNFB challenged animals served as controls (□; $n=2$). The DB-1 treated animals were compared both to those treated with G11 ($P<0.01$, 0·001 and 0·001 at 24, 48 and 72 h respectively) as well as to the untreated, sensitized animals (corresponding P values less than 0·01, 0·05 and 0·001).

Antibody treatment in vivo

The sensitized rats, age and sex matched, were divided into three groups. (For convenience this experiment was identically performed at two different occasions; this is why the numbers of rats, n, are given as $x+y$.) Immediately before DNFB challenge the rats in one group ($n=8+8$) were given one intraperitoneal (i.p.) injection of 1 mg DB-1 in 1 ml PBS. The second group of animals ($n=8+7$) was similarly injected with 1 mg of G11 in 1 ml PBS and the third group ($n=6+5$) was left untreated. After the final measurement at 72 h the rats were killed with ether. The ears were then cut off at the bases, immersed in Histocon (HistoLab., Bethlehem Trading Ltd. Gothenburg, Sweden) at +4°C (for maximally 2 h), snap frozen in chilled isopentane and then stored at −70°C.

Immunohistochemical staining

Acetone-fixed cryostat sections, 6 μm thick, were processed for peroxidase-anti-peroxidase (PAP) staining by the method of Sternberger (1979). The sections were cut through the centre of each ear extending from the top to the base. Mouse MoAbs, clones OX6 and OX17 (Ig content > =2 mg/ml) were obtained from Sera-Lab (Cambridge, UK). OX6 antibodies (IgG1, diluted 1:2000) have been shown to react with constant region determinant of rat class II antigens corresponding to I-A coded molecules in mice (McMaster & Williams, 1979), whereas OX17 antibodies (IgG1, diluted 1:1000) recognize a monomorphic determinant on the α chain of rat class II antigens, homologous with the mouse I-E product (Fukumoto, McMaster & Williams, 1982). Goat anti-mouse IgG was adsorbed on Sepharose-coupled rat IgG and used as a secondary antibody (diluted 1:10). Preformed complexes of horse-radish peroxidase and

Table 1. Expression of class II antigens on keratinocytes determined by immunoperoxidase staining 72 h after ear challenge.

Treatment		Expression on keratinocytes* OX6 antigens (n)†	OX17 antigens (n)†
None—normal skin		0 (5)	0 (5)
Unsensitized	DNFB challenge	0 (2)	0 (2)
Sensitized, no injection	DNFB challenge	+ + (2)	+ (3)
		+ + + (3)	+ + (2)
Sensitized, G11	DNFB challenge	+ + (7)	+ (8)
		+ + + (8)	+ + (7)
Sensitized, DB-1	DNFB challenge	0 (2)	0 (14)
		+ (13)	+ (2)
		+ + + (1)	

* Graded as 0 = undetectable, + = single groups of keratinocytes, + + = continuous and covering the basal layer or + + + = continuous and extending throughout the whole epidermis.

† Number of animals.

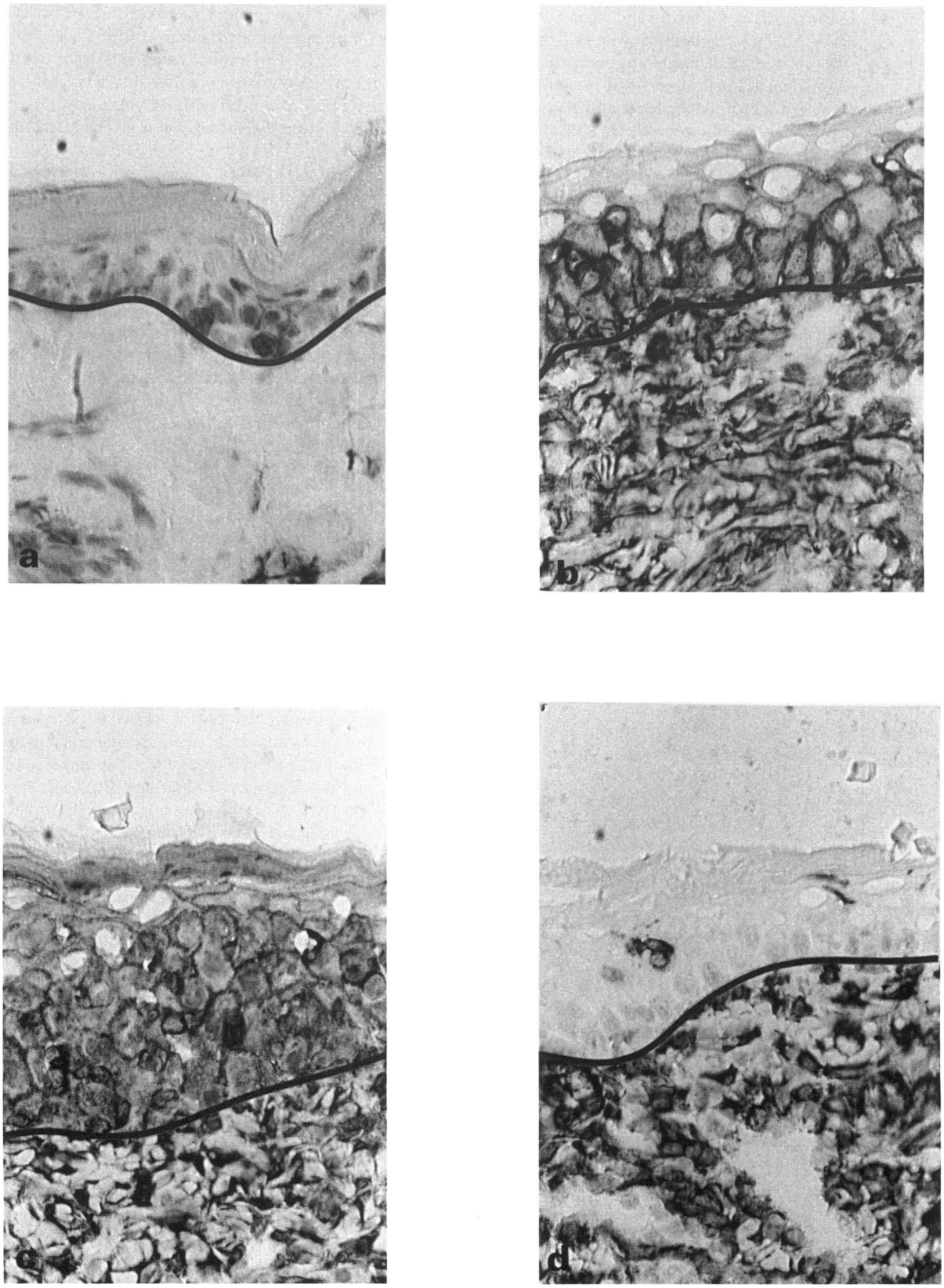

Fig. 2. Immunoperoxidase staining of OX6 reactive cells on frozen sections of rat ear skin 72 h after DNFB challenge. (a) An unsensitized animal. (b) A sensitized animal, (c) A sensitized animal given MoAb G11 i.p. prior to challenge. (d) A sensitized animal given MoAbs against IFN-γ, DB-1, i.p. prior to challenge. Note the expression of class II antigens of keratinocytes in (b) and (c). The sections were counterstained with haematoxylin. The solid line indicates the epidermal basal membrane zone.

monoclonal anti-horse-radish peroxidase antibody (diluted 1:500) were obtained from Dakopatts (Copenhagen, Denmark). The peroxidase reaction was developed with carbazole (Kaplow, 1975) and the sections counterstained with haematoxylin. The dilutions of the antibodies were determined using sections from normal lymph nodes and skin. Controls without the primary antibodies or with irrelevant antibodies gave no staining. The expression of class II antigens on the keratinocytes was graded from 0 to +++, where 0 means undetectable, + single groups of keratinocytes, ++ continuous and covering the basal layer and +++ continuous and extending throughout the whole epidermis. Keratinocytes were considered positively stained when the full circumferences of the cells were labelled. Each ear was also processed for haematoxylin and eosin staining.

Statistical analysis

The statistical significance of differences in the means for each experimental group was calculated with Student's *t*-test.

RESULTS

Effects of antibody treatment on the DTH-reaction

The animals treated with DB-1 showed a larger ear swelling after DNFB challenge compared with the other two sensitized groups. As seen in Fig. 1 the differences are statistically significant at each time point. Animals given the irrelevant antibody, G11, had a similar increase in ear swelling as the untreated group.

Expression of class II antigens in the epidermis

When assessing the expression of class II antigens in the epidermis at the challenge sites at 72 h, unsensitized but ear-challenged animals did not differ from normal rats, i.e. only dendritic Langerhans' cells expressed class II antigens (Table 1 and Fig. 2a).

Both animals not given any antibodies and those treated with G11 i.p., revealed, besides the class II antigen expression on Langerhans' cells, a ++ or +++ graded amount of OX6 positive keratinocytes. (Table 1 and Figs 2b and c). An induced expression of OX17 antigen on keratinocytes was also seen, although to a lesser extent (Table 1).

In contrast, the DB-1 treated animals with one exception, displayed an OX6 positive staining on the keratinocytes that was detectable only in a few foci +, or was even undetectable 0 (Table 1 and Fig. 2d). The expression of OX17 on keratinocytes was even less pronounced than that of OX6 (Table 1). The dendritic Langerhans' cells in the epidermis expressed both OX6 and OX17 antigens.

Dermal cell infiltrate

The dermal cell infiltrate as judged from haematoxylin and eosin-stained sections showed no marked difference between the DB-1 treated animals and the two other sensitized groups. The majority of the infiltrating cells in all three experimental groups expressed class II antigens.

There was no difference between normal rats and unsensitized rats that were ear challenged.

DISCUSSION

This study shows that *in vivo* treatment of rats with DB-1, a MoAb against rat IFN-γ, enhances the development of a DTH reaction. Furthermore, this treatment can inhibit the expression of class II transplantation antigens on keratinocytes that normally is induced during the DTH reaction (Suitters & Lampert, 1982; Scheynius & Tjernlund, 1984).

The latter inhibitory effect of DB-1 treatment seems logical, since IFN-γ is a well-known inducer of class II antigens on keratinocytes (Basham *et al.*, 1984; Scheynius, Johansson & van der Meide, 1986). According to initial experiments this effect is dose dependent; 0·5 mg of DB-1 i.p. resulted only in a moderate inhibition (data not shown), compared to the 1 mg used in this study. There was always a correlation between the enhanced DTH reaction and the diminished expression of class II antigens on keratinocytes. Thus, the only animal out of 16 in the DB-1 treated group that still revealed a strong class II antigen expression on keratinocytes (Table 1) also had less pronounced ear swelling. This discrepancy between treatment and class II expression may possibly be due to a technical failure during the injection. The presence of OX17 reactivity, corresponding to mouse I-E antigens, on keratinocytes was always less pronounced than OX6 (I-A) staining in accordance with a previous report on rats given recombinant rat IFN-γ intradermally (Scheynius *et al.*, 1986). Class II expression on Langerhans' cells seemed, however, unaffected by DB-1 treatment as judged by the presently used immunoperoxidase technique, emphasizing the constitutive character of the expression in these cells. Our observation is in agreement with that of Groenewegen, Buurman & van der Linden (1985) where cyclosporin A inhibition of IFN-γ secretion in dogs lead to abrogation of class II antigen expression on endothelial cells but did not affect that of Langerhans' cells.

It has clearly been demonstrated that epidermal Langerhans' cells can act as antigen-presenting cells for the stimulation of T lymphocytes and as allogeneic T-cell activators (Stingl *et al.*, 1978; Braathen & Thorsby, 1980; Scheynius *et al.*, 1982). Whether keratinocytes with induced expression of class II antigens contribute to immune regulation is still unclear. Quantitative variations in class II antigen expression can, however, play a central role in immune regulation (Janeway *et al.*, 1984). The observation that an excess of class II molecules can inhibit T-cell proliferation (Matis *et al.*, 1983) supports the notion of a possible suppressive action by the induced class II antigens on keratinocytes in a DTH reaction (Scheynius & Tjernlund, 1984). The enhanced DTH reaction observed after DB-1 treatment may thus be explained by the diminished induction of class II antigens on keratinocytes.

Induction of class II antigens is however not the only function of IFN-γ. This lymphokine seems to be equivalent to the macrophage-activating factor (MAF) (Schultz & Kleinschmidt, 1983) which is an important differentiation factor for macrophages (Collart *et al.*, 1986). It has been shown that class II antigen expressing, inflammatory macrophages may inhibit T 'helper' cell functions (Klareskog *et al.*, 1985). Thus it is possible that treatment with DB-1 antibodies, thereby reducing the levels of IFN-γ, may have abrogated the development of suppressive macrophages leading to an enhanced DTH reaction. Another possible mechanisms is that the ability to induce 'suppressor' T cells is disturbed by blocking the activity of IFN-γ (Noma & Dorf, 1985).

In conclusion, treatment with MoAbs directed against IFN-γ inhibits the class II antigen expression on keratinocytes during the DTH reaction suggesting that the local levels of IFN-γ were

reduced. The observed enhanced DTH reaction indicates that IFN-γ in certain conditions may play a self-limiting role in the immune response.

ACKNOWLEDGMENTS

Excellent technical assistance was given by Catharina Johansson and Hillevi Nilsson. This work was supported by grants from the Swedish Work Environment Fund, the Swedish Medical Research Council, the Edward Welander foundation and the Swedish Society of Medical Sciences.

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