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The Effect of Grenz Rays on Irritant Skin Reactions in Man

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To investigate the effect of grenz rays on irritant contact reactions, eleven healthy volunteers were studied. They were given 3 Gy of grenz rays, once a week for 3 weeks, to a defined area of the back. Twenty-four hours after the last treatment, serial dilution sodium lauryl sulphate patch tests were applied both on the grenz ray treated area and on the untreated control skin. Biopsy specimens were taken from the irritant reactions both from the grenz ray treated area and from the control area and different cell populations in dermis and epidermis were identified by monoclonal antibodies (Leu 2, 3, 4, 7, Leu M1, B1, OKT6). In the grenz ray treated epidermis there was a pronounced reduction of OKT6-positive cells but the composition of the dermal cellular infiltrate did not differ between control and grenz ray treated skin. The assessment of the patch test reactions did reveal a tendency towards weaker reactions in the grenz ray pre-treated skin but this difference was not statistically significant. It is concluded that grenz rays do not have a marked effect on the elicitation of irritant reactions. (Received May 13, 1986.)

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During the past decade it has been established that the epidermal Langerhans' cells (LC) play a central role in allergic contact dermatitis (ACD) because of their capacity of binding and presenting antigens. The surface density of functional LC seems to be a crucial factor for the induction of sensitization (1, 2).

In a recent report it was demonstrated that the number of OKT6-positive cells (hereafter regarded as Langerhans' cells, LC) was reduced in skin treated with grenz rays (ultrasoft X-rays, Bucky rays) (3). Further electron microscopic studies (4) have shown that this reduction reflects a true disappearance of LC. It has also been found that treatment of the skin with grenz rays prior to challenge with nickel prevents the development of ACD in patients with an established contact allergy to nickel (5). This suggests that LC might be required for the expression phase of the ACD. Another possible explanation for the effect of grenz ray treatment on ACD could be that the treatment causes a non-specific suppression of the inflammatory response in the skin. This hyhothesis is tested in the present investigation by determining whether grenz ray therapy can suppress the elicitation also of irritant contact reactions. For this purpose we used sodium lauryl sulphate to produce irritant contact reactions in skin treated with grenz rays as well as in untreated skin. Monoclonal antibodies were employed to identify the OKT6 positive cells and to characterize the cellular infiltrates.

MATERIAL AND METHODS

Subjects

Eleven Caucasians, five females and six males without clinical or anamnestic signs of skin disease, were included in the study. All had given their informed consent. Age range 23–56 years.

Grenz rays

The grenz ray machine factors were 11 kV, 20 mA, and a half-value layer of 0.03 mm Al. Focus-skin distance was 20 cm. There was a beryllium window.

Irritant reactions

Irritant reactions were produced by patch testing (Finn chambers) with different concentrations of sodium lauryl sulphate (SLS) in distilled water.

Experimental procedure

Each subject was first exposed to grenz rays, 3 Gy once a week for 3 weeks, in one and the same area of 15×15 cm on the right side of the back. Twenty-four hours after the last treatment identical SLS patch test were applied on the site of the grenz ray treatment and symmetrically on the untreated side of the back.

In a first group (3 subjects) we used 0.5, 1.0 and 2% SLS solutions and in a second group (8 subjects) we applied 0.5, 1.0, 2, and 5% SLS solutions. The test patches were left in place for 48 h and the reading of the test reactions was done 72 hours after the application of the tests. In two subjects in the first group the corresponding times were 24 and 48 h. The tests were graded according to the scoring system recommended by the International Contact Dermatitis Research Group (6).

Biopsies

At the time of reading of the test reactions two punch biopsies (diameter 3 mm) were taken from each subject after anesthesia with lidocain without epinephrine. One biopsy was taken from the strongest positive test reaction both in the untreated and in the grenz ray treated skin. Each specimen was immediately frozen on solid carbon dioxide and stored at −80°C until used. The specimen were cryostat sectioned at 6–8 μm and mounted on cooled glass slides.

Table I. *Irritant reactions produced by sodium lauryl sulphate in patch tests applied on grenz ray treated skin (GR) and on untreated control skin (CONTR)*

−=negative reaction, +=erythema, ++=oedema or vesiculation, +++=bullous or ulcerative reaction. ND=not done

			Sodium lauryl sulphate patch tests			
Subject	Age/sex	Treatment	5%	2%	1%	0.5%
1	31/F	CONTR	ND	+	+	+
		GR	ND	(+)	−	−
2	39/M	CONTR	ND	+	+	+
		GR	ND	(+)	−	−
3	32/M	CONTR	ND	+	+	−
		GR	ND	(+)	(+)	−
4	29/M	CONTR	++	+	+	−
		GR	++	+	+	−
5	33/M	CONTR	++	+	+	+
		GR	++	+	+	−
6	28/M	CONTR	+++	+	+	+
		GR	+++	+	+	+
7	27/F	CONTR	++	+	+	+
		GR	−	−	+	−
8	37/F	CONTR	+	+	(+)	−
		GR	+	−	−	−
9	56/F	CONTR	++	+	+	(+)
		GR	++	+	+	−
10	27/M	CONTR	++	+	−	−
		GR	+	−	−	−
11	31/F	CONTR	++	(+)	+	+
		GR	−	+	−	+

Monoclonal antibodies

The following Leu (Becton-Dickinson) (7, 8, 9), OKT (Ortho) (10), and B1 (Coulter Immunology) (11) mouse antihuman monoclonal antibodies were used: Leu 2 (cytotoxic/suppressor T-cell subset), Leu 3 (inducer/helper T-cell subset), Leu 4 (all T-cells), Leu 7 (killer cells), Leu M1 (monocytes and null cells), OKT6 (Langerhans' cells and thymocytes), B1 (all B cells). The specificity of these antibodies has been reviewed previously (12).

The sections were rinsed in phosphate buffered saline solution, incubated in normal swine serum and then overlaid with the monoclonal antibody at a dilution of 1:200 (Leu M1), 1:100 (OKT6 and B1), 1:40 (Leu 2 and 7), and 1:20 (Lue 3 and 4). Secondary incubation was performed with horseradish peroxidase-conjugated rabbit anti-mouse immunoglobulin antibody (Dako). Peroxidase activity was visualized by incubation with 3-amino-9-ethylcarbazol, AEC, and finally the sections were counterstained with Mayer's haemalum. The primary antibody was omitted in control sections for each reaction. As positive controls we used tonsils.

Characterization of cell populations

Sections were stained with the monoclonal antibodies indicated above. The proportions of infiltrating cells were estimated semi-quantitatively at ×500 magnification. In the case of OKT6, only dendritic cells with visible nucleus were regarded as positive, isolated dendrites were not scored.

Statistical analysis

For each concentration of SLS the Sign test was used to compare the reactions in the grenz ray treated and the untreated skin.

RESULTS

Patch testing

The results are summarized in Table I. In the grenz ray treated areas there was a slight decrease of the test response as compared with the tests in the untreated skin. However, this decrease was not statistically significant ($p>0.05$). The grenz ray treatment resulted in a slight pigmentation of the skin in all volunteers.

Immunohistologic characterization of cell populations

The results are summarized in Table II. The cell populations of Leu 2, Leu 3, Lue 4, Leu 7, Leu M1, and Leu B1 occurred in about the same proportions in the cellular infiltrates

Table II. *Immunohistologic characterization of the cellular infiltrate in irritant reactions in grenz ray treated and in untreated skin in eleven subjects*

0=no cells, 1=few, scattered, 2=moderate number, 3=large number of cells

Phenotype	Irritant reactions in	
	Grenz ray treated skin	Untreated skin
Leu 2 (T suppr/cytotox)	1–2	1–2
Leu 3 (T helper/induc)	2–3	2–3
Leu 4 (pan T)	1–3	2–3
Leu 7 (NK cells	0–1	0–1
B1 (pan B)	0–1	0–1
Leu M1 (monocytes)	1–3	2–3
OKT6 (Langerhans' cells, thymocytes)		
Epidermis	1	3
Dermis	0–1	0–1

found in the grenz ray treated skin and in the untreated skin. Compared with the positive reactions in the untreated skin the number of epidermal OKT6 positive cells was greatly reduced in the reactions in the grenz ray treated skin. The dermal cell infiltrate consisted mainly of T-lymphocytes (Leu 4) with T-helper/inducer cells (Leu 3) more prevalent than T-suppressor/cytotoxic cells (Leu 2). In the cell infiltrate we also identified mononuclear/null cells (Leu M1) and occasional NK-cells (Leu 7), B-lymphocytes (Leu B1) and OKT6 positive cells.

In the light microscope it was not possible to reveal any differences in the thickness of stratum corneum in the patch tests in grenz ray treated skin compared with untreated skin.

DISCUSSION

In the present study we have found that pre-treatment with grenz rays does cause a certain reduction of the expression of irritant reactions caused by sodium lauryl sulphate when the strength of the reactions was assessed by the naked eye. This reduction was not found to be statistically significant in this material. It has previously been demonstrated (13) that PUVA treatment causes a non-specific reduction of the skin response to irritant stimuli. This effect of PUVA was considered to be due to an increased thickness of stratum corneum with a reduced penetration of the irritant substance. It cannot be excluded that the penetration barrier is altered by the grenz ray treatment but we could not, however, detect morphological differences between the irritant reactions in the pre-treated and in the control skin. Another and a more probable explanation is that the grenz rays induced pigmentation of the skin is sufficent to influence the assessment of positive patch tests to such an extent that it could explain the difference noted in our experiments. The possibility that pre-treatment with grenz rays might cause a non-specific suppression of the inflammatory response at the irritant reactions was analysed by using a panel of monoclonal antibodies for the characterization of the dermal infiltrate. We found that the composition of the dermal cell infiltrate was the same in the strong and the weak patch test reactions in both the grenz ray treated and the untreated skin. The distribution of the phenotypes in the cell infiltrate was also in good accordance with that previously found in ACD in our laboratory (5). Previously immunohistological studies (14–17) have also shown that there are no major differences between the dermal cellular infiltrate in irritant and allergic contact reactions. Our results are also in agreement with these earlier works regarding the dominance of T-lymphocytes in the dermal cell infiltrates with a prevalence of T-helper/inducer cells (16, 17). Our results do not support the idea that the tendency towards weaker test reactions in the grenz ray treated skin is the expression of a non-specific suppression of the inflammatory response.

In contrast to the findings in the present study it has been shown that pre-treatment with grenz rays is capable to inhibit the clinical expression of ACD almost totally (5). Such a difference can be explained by the effect of grenz rays on the epidermal LC. The irritant reaction is a non-specific response to a harmful stimulus elicited by the direct toxic effect on the cells and tissues of the skin and do not involve the immunological system (18). The elicitation of ACD is instead a strictly regulated and antigen specific event requiring the presence of functional antigen presenting cells. Both in our previous (5) and in the present work the number of LC had been reduced in epidermis by the same dose of grenz rays. The combined data from these two studies suggest that the LC are required for the elicitation of ACD but not for the elicitation of irritant reactions. These data do not exclude the possibility that grenz rays may have an effect on already developed inflammatory processes via an influence on other levels than the antigen presenting cells.

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