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Brief Note

The Effect of Freeze-Thawing Treatment of Antigen on the Induction of Contact Sensitivity and Its Suppression

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Accodring to the concept, the hapten applied to the human or animal skin enters the skin where it binds epidermal constituents and becomes a complete antigen. This complete antigen is recognized by antigen-inexperienced T lymphocytes (initiator T cell). The recognition by initiator T cells requires the initial uptake and processing by macrophage-like antigen presenting cells (APC) such as epidermal Langerhans cells that presents immunologically relevant moieties to the T cells. These cells, after being stimulated by the antigen, proliferate and differentiate into effector T cells, and the organism in consequence becomes hypersensitive to the hapten. Most recently, Granstein *et al.*[1] have reported that murine epidermis also contains APC that is recognized for introduction of immunological unresponsiveness. It is considered that a certain population of APC stimulates initiator T cell and another population activates suppressor T cell by presenting the antigenic determinant in a suitable form. The object of the experiment reported here is to investigate the nature of antigens which induce contact sensitivity (CS) or unresponsiveness.

JY1 strain guinea pigs were painted with 0.2 ml of 5% 2,4-dinitrochlorobenzene (DNCB) ethanol solution on ear skin and ears were obtained from the animals 3 hours after painting. Epidermal cell suspensions (DNP-EC) were prepared from the ear skin according to a technique described previously.[2] A part of DNP-EC was frozen at −70°C and melted at room temperature three times. Dinitrophenylated ovalbumin (DNP-OVA) was prepared as described previously.[3] Dorsal and ventral surfaces of both sides of ears were stripped by repeated applications of cellophane tape (20 times). 5×10^6 of untreated DNP-EC, DNP-EC treated by freeze-thawing or 1.0 mg of DNP-OVA in PBS (0.01 M phosphate buffer saline, pH 7.2) was injected subcutaneously through normal or stripped ear skin of JY1 guinea pigs immediately after stripping. Fourteen days after injection of the antigens, skin test was performed by applications of 0.01 ml of 0.1, 0.05 and 0.025% DNCB ethanol solution on the depilated flank. The contact reactions were read 24 hours later and evaluated as described previously.[3] The degree of sensitivity was expressed as the total of all these readings in each animal. To assess whether animals were tolerant, animals that had showed little or no hypersensitivity to DNCB were painted with sensitizing dose of 5% DNCB ethanol solution (0.02 ml) on the shaved nape skin after testing. Seven days later, the other flank was challenged and contact reactions were read as above.

Contact sensitization to DNCB was achieved in the animals by subcutaneous injection of untreated DNP-EC (Group 1 in Table). The ability to induce

岡　大介，中川昌次郎，植木宏明

TABLE. Assessment of tolerance in the hyposensitive guinea pigs sensitized with DNP-EC or DNP-OVA.

Animal group	Sensitization	Treatment	Contact sensitivity to DNCB			Resensitization	Contact sensitivity to DNCB		
			Positive		Mean intensity		Positive		Mean intensity
			0.1%	0.05%			0.1%	0.05%	
1	DNP-EC	none	7/7	3/7	1.1	none			
2	DNP-EC freeze-thawing	none	2/7	0/7	0.2	DNCB	7/7	7/7	1.4
3	DNP-EC freeze-thawing	stripping	0/7	0/7	0	DNCB	3/7	0/7	0.5
4	DNP-OVA	stripping	0/7	0/7	0	DNCB	7/7	7/7	2.3
5	none	none				DNCB	7/7	7/7	1.8

CS was almost lost when DNP-EC was treated by freeze-thawing (Group 2). However, the treated DNP-EC was capable of producing unresponsiveness when the animals were injected subcutaneously through stripped skin and given subsequently application of DNCB to normal skin (Group 3). This suggests that antigenic determinant to activate suppressor T cell is different from that to stimulate initiator T cell. DNP-OVA given through stripped skin had no effect on subsequent sensitization with DNCB (Group 4). It has been suggested that tolerance can be induced only by DNP in association with self membrane.[4] However, Ptak *et al.*[5] have shown that there is no requirement for major histocompatibility complex identity between the haptenated cells and the host for specific immunosuppression to occur. Further analysis of antigens for induction of CS and tolerance must be done.

Daisuke OKA, Shojiro NAKAGAWA and Hiroaki UEKI

Department of Dermatology, Kawasaki Medical School, Kurashiki 701-01, Japan

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