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LYMPHOCYTE RECEPTORS FOR AUTOANTIGENS, AUTOLOGOUS SERUM INHIBITS SELF-RECOGNITION

By
H. WEKERLE, I. R. COHEN
and
M. FELDMAN

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Lymphocyte Receptors for Autoantigens, Autologous Serum inhibits Self-recognition

IMMUNE reactions are triggered by the recognition of an immunogen by specific lymphocytes. Autoimmune reactions are evoked when lymphocytes recognize autologous antigens, and react against them. The absence of reactivity against self-antigens in normal organisms is attributed either to the lack of self-recognizing lymphocytes[1] or to a regulatory mechanism which prevents potentially self-reactive lymphocytes from producing autoimmune reactions. The results of previous studies in our laboratory seemed incompatible with the first hypothesis, which is based on the absence of cells recognizing self-antigens. We demonstrated that lymphocytes from normal adult rats can be readily sensitized *in vitro* against syngeneic embryonic fibroblasts[2] as well as against syngeneic adult thymus reticulum cells[3]. In both cases the autoimmune reactions were induced in cell culture and the effector phase was measured either *in vitro* by assaying cell mediated cytolysis, or *in vivo* by the graft versus host (GvH) reaction[4]. These studies thus indicate that cells capable of reacting against self-antigens do exist in a normal population of lymphocytes. Two questions then arise. First, can one demonstrate that such lymphocytes do in fact possess specific receptors for self-antigens? Second, what prevents *in vivo* immune reactions against self-antigens?

To answer the first question we used cell monolayers as specific immunoadsorbents for the analysis of the initial recognition phase in cell-mediated immunity[5,6]. This approach has been used in our laboratory to analyse lymphocyte receptors for antigens of allogeneic and xenogeneic mouse cells. It was based on plating lymphocytes on monolayers of mouse fibroblasts. The lymphocytes which adhered to the monolayers were separated from those which did not. The first ones became highly sensitized and manifested specific lytic effect against the adsorbing fibroblasts. On the other hand, the nonadhering lymphocytes were incapable of becoming sensitized against fibroblasts of an H-2 phenotype identical with the adsorbing cells, although they did retain full reactivity against fibroblasts of an unrelated H-2 phenotype. Hence, adherence was based on the binding of lymphocytes via their cell receptors to the antigens of the fibroblast cell surface and therefore recognition *per se* can be expressed by the quotient of the cytotoxicity of the adhering and the nonadhering lymphocyte

populations (the coefficient of adherence). To test for the existence of receptors for self-antigens, the same procedure was applied to syngeneic combinations of lymphocytes and fibroblasts.

The following experiment was carried out. Lewis rat lymph node cells were suspended for 30 min in 50% horse serum and the lymphocytes were washed and plated on monolayers of syngeneic fibroblasts. After 3 h incubation the nonadhering cells were separated from those which firmly adhered to the fibroblasts (about 5%) and were transferred for sensitization to fresh Lewis monolayers. After 5 days in the sensitizing cultures the nonadherent groups were transferred to ^{51}Cr-labelled Lewis target monolayers to assay the extent of lysis. The result (Table 1) was that the lymphocytes developed from the adherent fraction manifested a cytolytic activity which was 4.5 times higher than that produced by the nonadherent cells. This coefficient of adherence demonstrates that Lewis lymphocytes possess receptors for self (Lewis) antigens. The adherence to the surface antigens of synthetic fibroblasts seems to take place via such receptors, resulting in the elimination from the nonadherent fraction of self reactive cells.

What then prevents *in vivo* autoimmune reactivity? Because serum factors have been found to play a role in certain cases of specific immunological unresponsiveness[7], we tested the effect of autologous serum on self-reactive lymphocytes. The experiment was similar to the first one, with the exception that the rat lymphocytes were first pretreated with 50% fresh autologous serum for 30 min. The same separation assay was performed. The result (Table 1) demonstrated no difference

Table 1 Specific Recognition of Syngeneic Fibroblasts by Lewis Lymphocytes and its Abrogation by Lymphocyte Pretreatment with Autologous Fresh Serum

Exp.	Fibroblast	Serum used for pretreatment	% Specific lysis ± s.d. Adherent	Non adherent	Coefficient* of adherence	%Inhibition† of adherence
a	Lewis	HS	16.98±1.34	3.75±0.81	4.50	0
	Lewis	Lewis	12.78±1.08	10.35±0.05	1.23	93
b	Lewis	HS	11.73±1.10	7.35±0.41	1.60	0
	Lewis	BN	12.45±0.80	8.18±1.15	1.52	13
	Lewis	Lewis	9.38±0.53	8.62±1.08	1.08	87

Lewis rat lymphocytes were incubated in 50% horse serum (HS); fresh autologous Lewis serum (Lewis) or fresh BN rat serum (BN) for 30 min before being plated on Lewis fibroblast cultures for 3 h.

* Coefficient of adherence (CA) $= \frac{\text{lytic effect of adherent cells}}{\text{lytic effect of supernatant cells}}$

† % inhibition $= \frac{\text{CA (horse serum pretreated)} - \text{CA (rat serum pretreated)}}{\text{CA (horse serum pretreated)} - 1} \times 100$

between the cytotoxicity produced by the adherent and the nonadherent fraction. Pretreatment of the lymphocytes with allogeneic BN serum diminished only slightly the coefficient of adherence. Thus, autologous serum inhibits specifically recognition of self-antigens by lymphocytes.

It could be argued that serum of Lewis rats either paralysed the lymphocytes during the incubation phase and thus inhibited binding in general, or that it contained some nonspecific immunosuppressive factors[8]. Had this been the case, Lewis lymphocytes pretreated with Lewis serum should be incapable of recognizing also antigens of genetically unrelated origin. Table 2 shows, however, that Lewis lymphocytes pretreated with autologous serum can react against xenogeneic mouse fibroblasts. Hence, autologous serum appears to prevent recognition of self by acting specifically on receptors for self-antigens.

What are the serum blocking factors? Wegmann *et al.*[9] and Hellström *et al.*[10] were able to decrease cytotoxic action by pretreating fibroblast target cells with serum from tetraparental mice, or from animals rendered tolerant neonatally. They suggested that either antibody or antigen-antibody complexes are responsible for the inhibition. To test whether the serum factors that maintain natural tolerance in our system might be similar to those active in induced tolerance

Table 2 Recognition of Xenogeneic Fibroblasts by Lewis Lymphocytes Pretreated with Different Sera

Fibroblast	Serum used for pretreatment	% Specific lysis ± s.d. Adherent	Nonadherent	Coefficient of adherence
C3H *	HS	38.40 ± 1.10	19.09 ± 0.59	2.00
C3H	RS	48.47 ± 0.39	18.21 ± 0.50	2.65
C3H	Lewis	46.43 ± 1.09	15.95 ± 1.25	2.91

* After pretreatment with horse serum (HS), stored pooled rat serum (RS) or fresh Lewis serum, Lewis lymphocytes were plated for 1 h on C3Heb mouse fibroblasts.

Table 3 Recognition of Syngeneic Fibroblasts Pretreated with Autologous Serum by Untreated Lewis Lymphocytes

Fibroblast	Serum used for pretreatment	% Specific lysis ± s.d. Adherent	Nonadherent	Coefficient of adherence
Lewis	HS	31.24 ± 0.62	16.95 ± 1.49	1.85
Lewis	RS	35.42 ± 1.95	17.20 ± 1.83	2.08
Lewis	Lewis	36.78 ± 1.05	20.04 ± 0.01	1.83

Lewis fibroblasts were incubated with 50% horse serum (HS), stored pooled rat serum (RS) or fresh Lewis rat serum, before coincubation with Lewis lymphocytes for 3 h.

we pretreated only the fibroblast monolayers with autologous serum prior to plating them with lymphocytes for adherence. Self-recognition was not inhibited at all by such treatment (Table 3). Hence, the serum factors in autologous sera which prevent autoimmune reactions do not seem to be antibodies which mask surface antigens. On the other hand, we could inhibit the recognition of xenogeneic fibroblast antigens by membrane particles and solubilized membrane antigens (Wekerle *et al.*, in preparation). Therefore, although the data of our present study do not exclude completely antibody or antigen-antibody complexes from representing the blocking element, they seem more compatible with the notion that soluble antigens are the candidates for the factors inhibiting self-recognition, possibly by specific blocking of the receptors for self-antigens.

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H. WEKERLE *
I. R. COHEN
M. FELDMAN

Department of Cell Biology,
The Weizmann Institute of Science,
Rehovot, Israel

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* On leave from the Max Planck Institut für Immunbiologie, Freiburg, Germany.

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