Brief Definitive Report

ROLE OF CONTRASUPPRESSION IN THE ADOPTIVE TRANSFER OF IMMUNITY*

BY MICHAEL IVERSON,[‡§] WLODZIMIERZ PTAK,[¶] DOUGLAS R. GREEN,[‡] AND RICHARD K. GERSHON[‡§]

From the [‡]Yale University School of Medicine, Department of Pathology, New Haven, CT 06510; [§]Howard Hughes Medical Institute, Laboratory of Cellular Immunology, Yale University School of Medicine, New Haven, Connecticut 06510; and [¶]Copernicus School of Medicine, Department of Immunology, Cracow, Poland

An animal that has previously encountered and reacted to antigen usually possesses an immune system poised to vigorously react to another challenge of the same antigen. It is often the case, however, that the immune cells of the primed animal are incapable of function when injected into an untreated, naive recipient. This barrier to adoptive immune transfer first appears in mice around 14 d of age (1). If the immune lymphocytes are injected by a subcutaneous route, a temporary immunity may ensue (2). Treatment of recipients with low doses of irradiation (3) or cyclophosphamide (Cy) (4–6) may allow successful transfer. In each case, it appears that effective adoptive transfer of immunity requires that the recipient's suppressor cell circuits be bypassed or curtailed.

The ability of adoptively transferred immune cells to function in normal adult recipients, however, does not always require preparation of the host. For example, lymphoid cells from contact-sensitized animals have been shown to effectively transfer delayed-type hypersensitivity upon intravenous injection into normal adult animals (7). In this paper we report experiments designed to study immunoregulatory lymphocyte interactions that may account for the relative ease with which contact hypersensitivity can be adoptively transferred. The described interactions are related to previously reported immunoregulatory activities. These results are discussed in terms of our understanding of delayed-typed hypersensitivity and its regulation.

Materials and Methods

Mice. CBA/J mice of either sex were obtained from The Jackson Laboratory, Bar Harbor, ME.

Cell Separation Procedure Using Vicia Villosa. V. villosa lectin was purchased from E. Y. Laboratories, Inc., San Mateo, CA. The lectin was dissolved in citrate saline buffer, pH 5.5. The lectin can be stored as a stock solution of 0.5 mg/ml at 4°C for several months. ~5 ml of stock lectin solution was applied to 10-cm tissue culture grade petri dishes and incubated for 45 min at room temperature. The petri dish was washed three times with phosphate-buffered saline (PBS), then incubated with tissue culture medium plus 2% fetal calf serum (FCS) for 15 min at room temperature. Spleen cells were adjusted to 7.5×10^6 cells/ml in tissue culture medium containing 5% FCS. 10 ml of the cell suspension was

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added per coated petri dish and incubated for 45 min at 37°C. The nonadherent cells were gently removed. The residual nonadherent cells were removed with a gentle wash with tissue culture medium. The bound cells were removed from the plate by adding 5 ml *N*-acetyl-D-galactosamine, 1 mg/ml in PBS, to cover the bottom of the dish. The cells were incubated for 5 min at 37 C and then pipetted with a pasteur pipette.

Total cell recoveries from this procedure were >95%. The yield of nonadherent cells was 70–75% of the cells applied, whereas the adherent population yield was 25–30% when whole spleen cell preparations were used. When purified T cells were used, the yields were 90–95% and 5–10%, respectively.

Immunization. Donor mice were immunized by painting the shaved abdomen and four paws with 150 μl of a 5% solution of picryl chloride in ethanol/acetone (3:1).

Adoptive Contact Hypersensitivity Assay. 4 d after the donor mice were immunized they were sacrificed and a spleen cell suspension was made. After the cells were treated (see text) they were injected intravenously into groups of four to six 7-wk-old CBA/J mice. The optimal dose of cells—5×10^7 cells/recipient—required for the transfer of contact sensitivity was determined in a dose response experiment (data not shown). Cell equivalent doses were used with the unfractionated population as the reference point. The recipients ears were painted immediately after transfer with a drop of an 0.8% solution of picryl chloride in olive oil. The thickness of both ears of each recipient was measured before, and 24 h after, the antigen challenge. The values for swelling are the differences in ear thickness before and after challenge with antigen. Thus, each value reported in the tables is the mean and standard deviation of 8–12 measurements per group. Ear swelling measurements in recipients that were not injected with cells (negative controls) were subtracted from the measurements of the experiment groups.

Cyclophosphamide (Cy). Some recipient mice were treated with Cy at a dose of 20 mg/kg body weight 24 h before adoptive transfer of immune cells.

Antisera. The anti-Lyt-1.1 and the anti-Lyt-2.1 were kindly supplied by F. W. Shen, Sloan-Kettering Institute, New York, NY. The anti-I-J serum was kindly supplied by D. B. Murphy, Yale University. Spleen cells were incubated for 30 min at room temperature at 10^7 cells/ml of appropriately diluted antibody. They were then washed and incubated an additional 30 min at 37°C in diluted rabbit complement (prescreened for low background cytotoxicity).

Results

Several strategies were used in attempts to fractionate immune lymphocytes to determine those populations responsible for adoptive transfer of immunity. Immune spleen cells were treated with anti-Lyt-1.1 or anti-Lyt-2.1 plus complement and injected intravenously into naive recipients. The ears were painted immediately with picryl chloride and ear swelling was measured at 24 h. Results are shown in Table I. Cells treated with complement alone (line *1*) or anti-Lyt-2 plus complement (line *2*)-transferred effective immunity. Treatment with anti-Lyt-1 plus complement, however, completely abrogated the transfer (line *3*). Thus, immune Lyt-1 T cells contain any and all populations required for effective transfer of contact sensitivity. On the other hand, no detectable immunity could be adoptively transferred with Lyt-2^+ T cells.

Adoptive transfer by immune Lyt-1 T cells into naive, untreated animals was observed in several other experiments, two of which are shown in Table II (line *1*). It was further observed that treatment of the Lyt-1 T cells with anti-I-J antiserum plus complement removed the capability to transfer immunity to naive recipients (line *2*). That this was *not* the case was shown when the I-J^-, Lyt-1^+ T cells were injected into animals that had been pretreated with a low dose (20 mg/kg) of Cy (line *4*). Thus, removal of an I-J^+ cell from the Lyt-1 T cells had no effect upon immune transfer into Cy-treated recipients (line *3* vs. line *4*), but completely abrogated transfer into normal animals (line *1* vs. line *2*). These experiments demonstrated that I-J^+, Ly-1 T cells acted to protect the immune

TABLE I

Ability of Different T Cell Sets to Transfer Adoptive Immunity (Contact Hypersensitivity) into Syngeneic Unimmunized Mice

Adoptive transfer* with immune	24-h ear swelling (10^{-3} cm) in challenged recipients‖
(1) Unfractionated spleen cells (C′ only)	4.9 ± 0.25
(2) Ly-1 spleen cells‡	5.8 ± 0.70
(3) Ly-2 spleen cells§	0.0 ± 0.50

* Transferred 5×10^7 cells/mouse, i.v.

‡ Spleen cells treated with anti Lyt-2 plus C′ before transfer.

§ Spleen cells treated with anti Lyt-1 plus C′ before transfer.

‖ Four recipients per group were treated as described and tested for contact sensitivity. Ear swelling measurements from both ears of each recipient (8 measurements/group) were used to determine a mean and standard deviation. Ear swelling measurements obtained from recipients that were not injected with cells, negative controls, were subtracted from the values obtained from the experimental groups.

TABLE II

I-J$^+$ Cells Are Required for the Adoptive Transfer of Contact Hypersensitivity by I-J$^-$ Cells Unless the Recipients Suppressor Circuit is Inactivated with Cyclophosphamide

Adoptive transfer of immune T cells treated with:*	Treatment of recipient mice	24-h ear swelling (10^{-3} cm) in challenged recipients§	
		Exp I	Exp II
(1) C′ only	None	7.9 + 0.20	7.5 + 0.25
(2) Anti-I-J plus C′	None	1.4 + 0.81	1.0 + 1.03
(3) C′ only	Cy‡	7.5 + 0.27	6.1 + 0.25
(4) Anti-I-J plus C′	Cy	5.3 + 0.80	5.5 + 0.75

* Anti Lyt-2, plus C′, treated immune T cells were treated with anti-I-J serum, as shown, before transfer.

‡ The recipients were given 20 mg/kg cyclophosphamide 24 h before transfer.

§ Six recipients per group were treated as described and tested for contact sensitivity. Ear swelling measurements from both ears of each recipient (12 measurements/group) were used to determine a mean and standard deviation. Ear swelling measurements obtained from recipients that were not injected with cells, negative controls, were subtracted from the values obtained from the experimental groups.

transfer of I-J$^-$, Lyt-1$^+$ T cells into recipients possessing a Cy-sensitive inhibitory activity. On the other hand, the I-J$^+$ cell was not required for transfer into Cy-pretreated animals.

It has been shown that some, but not all, immunoregulatory cells bind the lectin *V. villosa* (8). The technique of dividing the lectin-binding cells from those that do not bind the lectin has the added advantage that both populations of cells are recovered. Both populations can be tested either separately or added together. Thus, the immunoregulatory function of each can be directly demonstrated.

The *V. villosa* lectin was bound to plastic dishes and used to separate the spleen cells from mice immunized with picryl chloride into adherent and nonadherent fractions. Fractions were injected into normal and Cy-pretreated recipients; swelling of painted ears was measured at 40 h. This experiment was done three times with similar results. The results of one such experiment are

TABLE III

V. villosa Adherent Cells Are Required for the Adoptive Transfer of Contact Hypersensitivity by V. villosa Nonadherent Cells Unless the Recipient's Suppressor Circuit Is Inactivated with Cyclophosphamide

Adoptive transfer of immune spleen cells	Treatment of recipients	Ear swelling (10^{-5} cm) in challenge recipients‖
(1) Unfractionated	None	6.6 + 0.25
(2) Unfractionated	Cy§	6.7 + 1.05
(3) Lectin nonadherent*	None	1.8 + 0.27
(4) Lectin nonadherent	Cy	6.4 + 0.55
(5) Lectin adherent‡	None	0.9 + 0.70
(6) Lectin adherent	Cy	0.8 + 0.63
(7) Nonadherent + adherent	None	6.0 + 0.41
(8) Nonadherent + adherent	Cy	6.5 + 0.84

* Nonadherent cells were gently washed from *V. villosa* lectin coated plates.

‡ Adherent cells were eluted from *V. villosa* lectin coated plates with 1 mg/ml *N*-acetyl-D-galactosamine for 10 min at 37°C.

§ The recipients were given 20 mg/kg cyclophosphamide 24 h before transfer.

‖ Five recipients per group were treated as described and tested for contact sensitivity. Ear swelling measurements from both ears of each recipient (10 measurements/group) were used to determine a mean and standard deviation. Ear swelling measurements obtained from recipients that were not injected with cells—negative controls—were subtracted from the values obtained from the experimental groups.

shown in Table III. Unfractionated cells transferred specific immunity to both normal and Cy-treated recipients (lines *1* and *2*). The *V. villosa* nonadherent fraction contained the immune population, evidenced by effective transfer into the Cy-treated animals (line *4*) but was incapable of transferring immunity to the normal animals (line *3*). The lectin-adherent cells were unable to transfer immunity to either group (lines *5* and *6*), but completely protected the transfer of immunity by the nonadherent fraction into normal recipients (line *7*).

We suggest, therefore, that the immune Lyt-1 T cells contained at least two subsets. One subset (I-J$^-$, *V. villosa* nonadherent) possessed immune function and was capable of transferring contact sensitivity to Cy-treated animals. The second population (I-J$^+$, *V. villosa* adherent) were incapable of transferring immunity alone; rather, their function was to allow the first subset to function in the suppressive environment of the normal recipient.

Discussion

These results suggest that I-J$^-$, *V. villosa* nonadherent immune Lyt-1$^+$ T cells from contact-sensitized donors are capable of transferring immunity on their own if the recipient animals are pretreated with a low dose of Cy. Several laboratories have demonstrated that low dose Cy treatment is effective in removing elements of suppressor T cell circuits (9–11). It is likely, therefore, that suppressor T cells are responsible for preventing adoptive transfer of the isolated immune population into normal recipients.

Unfractionated Lyt-1$^+$ cells from immune donors are fully capable of transferring immunity even in the face of the above suppressor cell activity. This ability is dependent upon the presence of I-J$^+$ and *V. villosa* adherent cells in the Lyt-1$^+$ T cell population. This population functions to protect the immune Lyt-1$^+$ T

cells (I-J$^-$, *V. villosa* nonadherent) from suppression, while having no direct immune capability themselves.

Both the biological function and the cell surface characteristics of the latter population are consistent with those of a previously described immunoregulatory T cell, the contrasuppressor effector cell (8, 12, 13). The contrasuppressor effector cell is an I-J$^+$, Lyt-1$^+$ T cell that adheres to the *V. villosa* lectin and functions to render helper T cells resistant to suppressor T cell signals.

We propose, therefore, that contact sensitization involves induction of both an immune T cell and a contrasuppressor effector T cell population. The latter is responsible for the ease of adoptive transfer of such immunity into normal animals. Physiologically, it may function to protect local immunity from systemic suppressive signals. Macher and Chase (14) demonstrated that removal of a sensitized area 4 h after painting resulted in the appearance of tolerance rather than immunity. Ptak et al. (15) showed that hapten presented on Langerhans cells of the skin induced immunity even in the presence of potent tolerogenic signals. Thus, the induction of contrasuppressor T cells by antigen presented on specialized antigen-presenting cells (such as Langerhans cells) could account for a number of features of contact sensitivity.

The presence of contrasuppressor T cells in immune populations used in transferring contact sensitivity suggests a slight paradox. Immune T cells can be readily suppressed by the action of a potent antigen-specific suppressor T cell factor (16). It is possible that the action of this suppressor factor in the presence of contrasuppressor cells is quantitative, that is, it overwhelms the immune cells. This suggests that the suppressive environment of the naive recipient is quantitatively and/or qualitatively less inhibitory than when preformed suppressor factor is used.

The observations made in this paper bear on one more point. Several laboratories have described I-J$^+$, Lyt-1$^+$ T cells that function to help B cells (17, 18). Given the experiments described herein, we suggest the possibility that in some cases an *apparent* I-J$^+$ helper cell may in fact be the result of an I-J$^-$ helper cell under the protection of an I-J$^+$ contrasuppressor cell. Use of the *V. villosa* lectin in fractioning such populations might prove most interesting (19).

Summary

The data presented in this paper show that the population of cells that adoptively transfer contact hypersensitivity are Lyt-1$^+$ 2$^-$, I-J$^-$ and nonadherent to *V. villosa* lectin. However, the adoptive transfer of immunity by this population of cells is successful only when the recipient has been treated in such a way as to impair the host immunosuppression mechanism. This population cannot, on its own, transfer immunity to adult, untreated naive recipients unless an additional population of immunoregulatory cells is present. This immunoregulatory population does not itself adoptively transfer immunity. This latter population is differentiated from the immune cells in that they are Lyt-1$^+$ 2$^-$, I-J$^+$ and are adherent to *V. villosa* lectin. Both populations are required to adoptively transfer immunity to adult untreated naive recipients.

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