Brief Definitive Report

THE *I-J* SUBREGION CODES FOR DETERMINANTS ON SUPPRESSOR FACTOR(S) WHICH LIMIT THE CONTACT SENSITIVITY RESPONSE TO PICRYL CHLORIDE*

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The cell-mediated immune reactivity (CMI) of mice to contact chemicals such as picryl chloride (PCl) is influenced by thymus-derived suppressor T lymphocytes (1, 2). The development of these suppressor T lymphocytes is stimulated by the intravenous administration of 2,4,6-trinitrobenzene sulfonic acid (TNBS). Zembala and Asherson have further demonstrated that a specific suppressor factor(s) can be detected in the supernates of cultured suppressor T cells. This factor suppresses the transfer of contact sensitivity (CS) to PCl (1, 2). In experiments reported elsewhere (3), we have shown that the PCl suppressor supernates of Zembala and Asherson can also suppress the development of contact sensitivity to PCl. The immunochemical analysis of suppressor factor (SF) operative in the CS response to PCl has revealed many similar properties (3) to other suppressive moieties functioning to limit the plaque-forming cell (PFC) response to dinitrophenylated-keyhole limpet hemocyanin (DNP-KLH) (4) or to synthetic copolymers (5). The comparable sizes of the molecular species as well as the strict antigen specificity of each respective suppressive factor, suggested that there might be a common origin of these substances. Indeed, in each case these respective factors were found to bear determinants controlled by the *H-2* gene complex (4, 5).

Recently, in selected systems, the *I-J* subregion has been found to code for the Ia determinants present on suppressor cells (6) and suppressor factors (4, 5). In accord with these findings, we report that antigen-specific SF which limit the CS response to PCl bear *I-J* determinants, implying that analogous suppressive regulatory mechanisms in CMI as well as antibody responses may be determined by genes of one subregion of the *H-2* complex.

Materials and Methods

Mice. CBA/J ($H\text{-}2^k$) female mice 8–10 wk of age were purchased from The Jackson Laboratory, Bar Harbor, Maine.

Antisera. The antisera used in these studies were (C57BL/10 × A.CA)F_1 anti-B10.Br, (3R × DBA/2)F_1 anti-5R, B10.D2, anti-B10.A, and (B10 × LP.RIII)F_1 anti-4R. The alloantisera were raised by 6–10 biweekly intraperitoneal injections of 2–5 × 10^7 spleen cells.

Antigens. TNBS and 2,4,6-trinitrochlorobenzene (PCl) were purchased from Eastman Kodak

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Co., Rochester, N. Y. To induce suppressor cells TNBS was dissolved in phosphate-buffered saline (PBS), pH 7.2, and adjusted to pH 7.4 by 1 N NaOH. 5 mg TNBS in a 0.1-ml vol was administered by the tail vein. Suppressor cells were obtained from various organs at day 5 after TNBS injection.

To immunize, 7% PCl in absolute alcohol was applied to the shaved abdomen in 0.1 ml and a total of 0.05 ml of that solution was applied to the foot pads. For challenge a 1% PCl solution in olive oil was applied to the left ear. The schedule for elicitation of CS was the application of 7% PCl at day 0 and challenge with 1% PCl solution to the ear on day 5.

Suppressor Factor(s) Production. On day 5 after TNBS injection, animals were challenged with 7% PCl. 18 h later lymph nodes and spleens were removed, aseptically, and single cell suspensions made by gently grinding the separate organs in chilled Hanks' solution using glass homogenizers. The cell number was determined and their viability assessed by the Trypan blue-dye exclusion technique. Cells were resuspended at a concentration of 1×10^7 cells/ml in Eagle's minimal essential medium supplemented with 10% fetal calf serum penicillin, 100 μ/ml glutamine, and streptomycin (50 μg/ml), were placed in Falcon Tissue Culture Flasks (Falcon Plastics, Div. Becton, Dickinson & Company, Oxnard, Calif.), and maintained at 37°C for 48 h in vitro. Supernates were gathered and concentrated by negative pressure dialysis in borate-buffered saline (PBS, pH 8). The supernates were adjusted to volumes equivalent to the product from 10^8 live cells/ml and frozen until used. Suppressor supernates were administered sequentially at a dose equivalent of 2×10^7 cells/day/mouse for 3 consecutive days beginning at the time of immunization.

Preparation of Reverse Immunosorbents. Reverse immunosorbents were prepared by the insolubilization of the globulin fraction of the heat-inactivated alloantisera to cyanogen bromide-activated Sepharose 4B by the method of Cuatrecasas (7).

Results

Suppressor Factor(s) are Antigen Specific. We have previously shown that suppressor cells elaborate suppressor factors which can be isolated from supernates in vitro (3). Using immunosorbents prepared by coupling trinitrophenyl-bovine serum albumin (TNP-BSA) to Sepharose, as shown in Table I, we have been able to specifically remove suppressive activity from such supernates. Furthermore, as additional confirmation of specific depletion of such factors, we have been able to recover the suppressive activity from these immunosorbents by pH 2.8 glycine HCl elution. These data, therefore, confirm previous observations by Zembala et al. (2) concerning the specificity of such factors.

Suppressor Factor(s) Operative in Limiting CS Responses are Linked to Determinants of Gene Products of the I-J Subregion of the H-2 Complex. In order to identify the precise origin of SF which limit the CMI response to PCl, suppressor supernates were passed through reverse immunosorbents coupled with the globulin fractions of the heat-inactivated alloantisera at 4°C and the residual suppressive activity was assessed by in vivo administration of the effluent. As can be seen from Table II, it was first established that anti-H-2^k alloantisera could completely deplete the supernates of any discernible activity. It was also established that B10.D2 anti-B10.A (anti-K^k plus I-A^k plus I-B^k plus I-J^k) could remove the suppressive molecules (data not shown). In order to clarify which *H-2* subregion in the *K* end of *H-2* coded for suppressive moieties, antisera to K^k, K^k plus $I\text{-}A^k$, or $I\text{-}J^k$ regions were used to prepare reverse immunosorbents. Only anti-I-J^k antisera could reproducibly and effectively remove suppressive activity. In all cases, only when the antisera contained antibodies directed at the $I\text{-}J^k$ subregion determinants was suppressive activity lost

TABLE I

Hapten-Specificity of Suppressor Factors Operative in the Development of Contact Sensitivity

Group*	Treatment	Immunization‡	Challenge§	Increment ear thickness,‖ 10^{-3} in. ± SEM	*P*
I	Suppressor cell supernate¶	+	+	1.4 ± .2	–
II	Suppressor cell supernate passed TNP-BSA-Sepharose	+	+	3.5 ± .6	<0.05
III	Suppressor cell supernate eluted from TNP-BSA-Sepharose (pH 2.8 glycine HCl)	+	+	1.5 ± .2	NS**
IV	Suppressor cell supernate passed BSA-Sepharose	+	+	1.7 ± .1	NS
V	–	+	+	3.7 ± .2	<0.001

* Each group contains five female CBA mice, 8–10 wk of age.
‡ Immunization, 7% PCl epicutaneously.
§ Challenge, 1% PCl on the left ear on day 5.
‖ Left ear thickness minus nonchallenged right ear thickness.
¶ 2×10^7 cell equivalents injected intravenously each day for 3 days, beginning at immunization.
** NS, not significant.

TABLE II

The I-J Subregion Codes for Suppressor Factor(s) Which Limit the Contact Sensitivity Response to Picryl Chloride

Group*	Supernate	Absorption‡	Immunization§	Challenge‖	Mean increment¶ ear thickness, 10^{-3} in. ± SEM	*P*
I	Suppressor spleen**	–	+	+	2.4 ± .2	–
II	" "	Anti-H-2^k Sepharose	+	+	4.1 ± .3	<0.008
III	" "	Anti-K^k + I-A^k Sepharose	+	+	2.6 ± .1	NS‡‡
IV	" "	Anti-K^k	+	+	2.6 ± .2	NS
V	" "	Anti-I-J^k Sepharose	+	+	4.0 ± .3	<0.006
VI	" "	Normal mouse immunoglobulin Sepharose	+	+	2.7 ± .4	NS
VII	" "	–	+	+	3.9 ± .2	<0.007

* Each group contains five female CBA mice, 8–10 wk of age.
† 1 ml of suppresor supernate from 3×10^8 cells was passed through immunosorbents, and after elution was concentrated to the original volume before injection.
§ Immunization, 7% PCl epicutaneously.
‖ Challenge, 1% PCl on the left ear on day 5.
¶ Left ear thickness minus nonchallenged right ear thickness.
** 2×10^7 cell equivalents injected intravenously each day for 3 days, beginning at immunization.
‡‡ NS, not significant.

on passage. We, therefore, conclude that at least certain elements necessary for suppression of CS bear specificities coded for by the *I-J*^{k} subregion.

Discussion

A variety of murine immune responses are controlled by genes of the *H-2* complex (8). The present observation that CS can be negatively regulated by a product of genes of the *I-J* subregion reinforces the notion that similar control mechanisms exist for CMI as for antibody responses. It has also been established that tumor antigen-specific suppressor factors which are *K*-end gene products of the *H-2* complex, can suppress the immune response to tumor antigen (9, 10).

This observation coupled with the data presented here lead to the conclusion that such *H-2* regulation is a substantial component of the biological control mechanisms of many CMI responses. We have been able to confirm and extend the observations of Asherson and Zembala (1) that suppressor supernates contain an antigen-specific suppressor factor, and that such factor(s) contain *I-J* determinants.

The extent of the I-J region's influence on the regulation of the immune response is currently being investigated in several laboratories. The data presented herein and in a companion paper (5) establishes that the *I-J* subregion, originally identified on cells mediating specific allotypic suppression (6) and on KLH-specific suppressor factor (4) controls a widely diverse range of specific suppressor responses.

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