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 (PC1) 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 PC1 (1, 2). In experiments reported elsewhere (3), we have shown that the PC1 suppressor supernates of Zembala and Asherson can also suppress the development of contact sensitivity to PC1. The immunochemical analysis of suppressor factor (SF) operative in the CS response to PC1 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 PC1 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-2k) 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)F1 anti-B10.Br, (3R × DBA/2)F1 anti-5R, B10.D2, anti-B10.A, and (B10 × LP.RIII)F1 anti-4R. The alloantisera were raised by 6-10 biweekly intraperitoneal injections of 2-5 × 10⁷ spleen cells.

Antigens. TNBS and 2,4,6-trinitrochlorobenzene (PC1) 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% PCI 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% PCI solution in olive oil was applied to the left ear. The schedule for elicitation of CS was the application of 7% PCI at day 0 and challenge with 1% PCI solution to the ear on day 5.

**Suppressor Factor(s) Production.** On day 5 after TNBS injection, animals were challenged with 7% PCI. 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 x 10⁷ cells/ml in Eagle's minimal essential medium supplemented with 10% fetal calf serum penicillin, 100 μg/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 10⁸ live cells/ml and frozen until used. Suppressor supernates were administered sequentially at a dose equivalent of 2 x 10⁷ 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 PCI, 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-2k alloantisera could completely deplete the supernates of any discernible activity. It was also established that B10.D2 anti-B10.A (anti-Kk plus IAk plus IBk plus I-Jk) 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 Kk, Kk plus IAk, or I-Jk regions were used to prepare reverse immunosorbents. Only anti-I-Jk antisera could reproducibly and effectively remove suppressive activity. In all cases, only when the antisera contained antibodies directed at the I-Jk subregion determinants was suppressive activity lost
on passage. We, therefore, conclude that at least certain elements necessary for suppression of CS bear specificities coded for by the I-J\(\kappa\) 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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References
1. Asherson, G. L., and M. Zembala. 1976. Suppressor T cells in cell-mediated immunity. Br. Med. Bull. 32:158.
2. Zembala, M., G. L. Asherson, B. Mayhew, and J. Krejci. 1973. In vitro absorption and molecular weight of specific T-cell suppressor factor. Nature (Lond.) 253:72.
3. Greene, M. I., A. Pierres, and B. Benacerraf. 1977. The specific suppression of contact sensitivity. Arthritis Rheum. In press.
4. Tada, T., M. Taniguchi, and C. S. David. 1976. Properties of the antigen-specific suppressive T-cell factor in the regulation of antibody response of the mouse. IV. Special subregion assignment of the gene(s) that codes for the suppressive T-cell factor in the H-2 histocompatibility complex. J. Exp. Med. 144:713.
5. Thèze, J., C. Waltenbaugh, M. Dorf, and B. Benacerraf. 1977. Immunosuppressive factor(s) specific for L-glutamic<sup>60</sup>-L-tyrosine<sup>60</sup> (GT). II. Presence of I-J determinants on the GT-suppressive factor. J. Exp. Med. 146:287.
6. Murphy, D. B., L. A. Herzenberg, K. Okumura, L. A. Herzenberg, and H. O. McDevitt. 1976. A new I subregion (I-J) marked by a locus (Ja-4) controlling surface determinants on suppressor T lymphocytes. J. Exp. Med. 144:699.
7. Cuatrecasas, P. 1970. Protein purification by affinity chromatography. J. Biol. Chem. 245:3059.
8. Paul, W. E., and B. Benacerraf. 1977. Functional specificity of thymus-dependent lymphocytes. A relationship between the specificity of T lymphocytes and their functions is proposed. Science (Wash. D. C.). 195:1293.
9. Greene, M. I., S. Fujimoto, and A. H. Sehon. 1977. The nature of immunosuppressor T cells and their factors in the tumor-bearing host. J. Supramol. Struct. 1(Suppl.):210.
10. Greene, M. E., S. Fujimoto, and A. H. Sehon. 1977. Regulation of the immune response to tumor antigen. III. Characterization of thymic suppressor factor(s) produced by tumor bearing hosts. J. Immunol. In press.