incidence of the exciting laser beam, with convergent focusing optics and spatial filtering and diode-array detection techniques. The ruby pressure scale can now be extended above 0.21 TPa because the fluorescence of diamond is diminished at higher pressures. The ruby fluorescence frequency shifts, measured at several points of increasing pressure in these experiments, correspond to pressures of 0.28 to 0.55 TPa on the extrapolated scale.

The disappearance of diamond fluorescence interference at pressures above 0.28 TPa is important in high-pressure experimentation because of the need to track the ruby fluorescence lines as they shift with pressure and to monitor the pressure above approximately 0.28 TPa. In the absence of strong diamond fluorescence interference, measurements of weak scattering of light, such as Raman or Brillouin spectra of solids (*2*), are also feasible to pressures of 0.55 TPa. Such spectroscopic measurements should yield quantitative information about the insulator-metal transition in hydrogen and other condensed matter at high compression. The results would be directly applicable to the understanding of the deep interiors of the earth and other planets.

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The Human Immune Response to the OKT3 Monoclonal Antibody is Oligoclonal

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The availability of highly specific and homogeneous antibodies to human T cells by the hybridoma technique has elicited new interest in the clinical use of antibodies to lymphocytes as immunosuppressive agents. OKT3 is the murine monoclonal antibody that has been the most widely used in clinical transplantation to induce immunosuppression. This antibody recognizes a membrane molecular complex, exclusively present on mature human T lymphocytes, which is tightly linked to the T-cell antigen receptor. The long-term therapeutic use of murine monoclonal antibodies in vivo is hampered by the intense antibody response that occurs in most human patients. Thus, when administered alone, OKT3 manifests its immunosuppressive activity only during the 10 to 15 days that precede the onset of sensitization. The results presented here show, by use of isoelectrofocusing, that the antibody response to OKT3, already reported to be restricted in its specificity (only anti-isotypic and anti-idiotypic antibodies are produced), is in addition oligoclonal. This restriction of the anti-monoclonal response may suggest that an efficient way to circumvent the sensitization problem would be to administer consecutively different monoclonal antibodies presenting the same specificity but distinct idiotypes.

MONOCLONAL ANTIBODIES HAVE been used successfully in vitro as highly specific probes. As therapeutic agents, however, they have not proved so successful (*1–6*). In studies of their antitumor or immunosuppressive effects in vivo, their ability to deplete the target cells (tumor cells or normal T cells) gave rise to an initial enthusiasm, but it was soon realized that there were two major obstacles to be circumvented before monoclonal antibodies could be used on a long-term basis. The first obstacle, antigenic modulation, may result in annihilation of the antibody therapeutic effect, when the aim of therapy is cell depletion and not merely the removal from the cell membrane of a functionally important molecule (*2, 4, 7*). The second obstacle, monoclonal antibody sensitization, occurs in most patients treated with the same monoclonal antibody for several consecutive days (*4, 6–9*). This sensitization is often intense and rapid and may abrogate the therapeutic effect of the monoclonal antibody and expose the patient to the risk of serum sickness. The results reported here suggest that the immune response to xenogeneic monoclonal antibodies is oligoclonal and for the most part specific for the antibody injected. This is interesting from the theoretical point of view since it illustrates in humans the notion that immunization with low doses of xenogeneic immunoglobulins administered intravenously induces a restricted immune response. It is also important clinically because it suggests a practical means of circumventing the sensitization side effect by shifting to another antibody with different characteristics.

Serum samples were collected from five renal allograft recipients treated prophylactically, that is, to prevent rejection, with the OKT3 monoclonal antibody (Ortho). As described elsewhere (*10*), these patients were participating in a randomized trial that included a total of 26 allograft recipients receiving OKT3 (5 mg/day injected intravenously for 13 to 30 days) either alone (six patients) or with azathioprine (3 mg per kilogram of body weight per day) and prednisone (0.25 mg/kg per day) therapy. The five patients tested showed intense antibody response against OKT3. In addition, the sera from four rhesus monkeys that received a skin allograft and that were prophylactically treated with OKT4 (Ortho) monoclonal antibody (5 mg/day for 10 to 16 days) (*11*) were also analyzed.

Analytical isoelectrofocusing was performed with thin-layer (0.5 mm) polyacrylamide gels (acrylamide, 5 percent weight to volume; bisacrylamide, 0.15 percent weight to volume; ammonium persulfate, 0.07 percent weight to volume), containing 0.08 percent (weight to volume) ampholytes *p*H 3.5 to 10 (LKB). The sera (10 μl diluted by 50 percent in saline) were loaded onto sample applicators and focused at a constant

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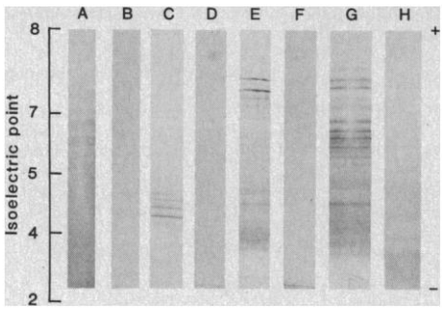

Fig. 1. (A–C) Sera from patient D.E.B. stained with (A) GAHu, (B) OKT3 and GAM (serum collected before OKT3 treatment), and (C) OKT3 and GAM (serum collected at day 30 of treatment). (D and E) Sera from patient G.U.I. stained with OKT3 and GAM. Serum was collected for (D) before OKT3 treatment and for (E), at day 20 of treatment. (F–H) Sera from patient Q.U.O. stained with (F) OKT3 and GAM (serum collected before OKT3 treatment), (G) OKT3 and GAM, and (H) GAM (serum collected at day 25 of OKT3 treatment).

current of 50 mA for 1 hour at 4°C, to a final 2000 V. The focused proteins were then immediately put in contact with nitrocellulose membranes (0.45 μm, Schleicher & Schuell) and electrophoretically transferred (Bio-Rad Trans-Blot cell) from the anode to the cathode at 4°C in 1 percent acetic acid for 1 hour at 30 V and 2 hours at 72 V. Nitrocellulose filters were then labeled (at room temperature) as follows: soaking for 30 minutes in phosphate-buffered saline, *p*H 7.4, containing 10 percent fetal calf serum and 0.2 percent Triton X-100 (PBS-Tx); incubation for 1 hour with the first antibody (OKT3, OKT3-Fab, OKT4, or OKT8; Ortho), 10 μg/ml in PBS-Tx; four washes in PBS-Tx; incubation for 1 hour with the appropriate dilution of peroxidase-labeled goat antiserum to mouse immunoglobulins (light chain–specific) (GAM, Institut Pasteur). As controls, filters were stained only with GAM or with peroxidase-labeled goat antiserum to human immunoglobulins (light chain–specific) (GAHu) (Nordic). Diaminobenzidine tetrahydrochloride (0.5 mg/ml in tris-HCl 50 m*M*, *p*H 7.6, containing 1 percent H_2O_2) was used as the peroxidase substrate.

The monoclonal antibody OKT3, which reacts with all human mature peripheral T cells, has been administered prophylactically to human renal allograft recipients either alone or together with azathioprine and low-dose steroids (*10*). In the two procedures used, immunosuppression has been achieved, as assessed by the disappearance of all circulating $OKT3^+$ cells and the absence of rejection episodes (*7, 9, 10*). Significant serum concentrations of OKT3 were present during treatment but rapidly waned as soon as patients' immunoglobulin (Ig) against the OKT3 monoclonal antibody (particularly of the IgG type) appeared (*7, 9, 10, 12*). Such antibodies were detected as soon as by days 9 to 11 when OKT3 was administered alone, but significantly later (days 20 to 30) and in lower titers when low dose conventional immunosuppressive treatment was administered concurrently to OKT3 (*7, 12*). Antibodies to OKT3 were detected by an enzyme-linked immunosorbent assay (ELISA), for which we used microplates coated with the patient sera and successive addition of OKT3 (in parallel with other murine monoclonal antibodies presenting different specificities or isotypes) and peroxidase-labeled GAM (*12–14*). The antibodies to the OKT3 molecule that were detected included antibodies specific to the murine IgG2 isotype: there was no reaction against non-IgG2 monoclonal antibodies (several IgG1, IgG3, and IgM molecules were screened). Moreover, purification of serum from immunized patients by consecutive chromatography on affinity columns with bound OKT8 (IgG2a) and OKT3 (IgG2a) demonstrated that the antibody response to OKT3 also included large proportions of anti-idiotypic antibodies reacting exclusively with OKT3 and its Fab or $F(ab')_2$ fragments (*12–14*).

To further investigate this restricted response, we performed isoelectrofocusing. Results obtained from all five patients tested (showing the most intense antibody response) indicated that the antibody response to OKT3 involved a limited number of clones (three to seven bands depending on the serum, located for each patient in a narrow range of isoelectric points) (Fig. 1). In most cases only a very weak polyclonal staining was detected with another IgG2a monoclonal antibody, namely OKT8 (Fig. 2). A restricted antibody response to OKT3 was observed previously in a patient who produced only anti-isotypic antibodies (*13*) (Fig. 3). These anti-isotypic antibodies did not accelerate OKT3 clearance in this patient since, even in the presence of these antibodies, high serum OKT3 concentrations were observed and no circulating $OKT3^+$ cells (*13*).

These results are not unique to OKT3 since similar data have been obtained for both isotype-idiotype restriction and oligoclonality with sera of rhesus monkeys treated with various monoclonal antibodies to T cells (*11, 15*) (Fig. 4).

These data demonstrate that the in vivo response to xenogeneic monoclonal antibodies may be restricted and thus may open new possibilities for overcoming the sensitization problem. The strategies that might be used include association with conventional immunosuppressive treatment (with the risk of cumulative immunosuppression), induction of tolerance to murine Ig (with the risk of sensitization), use of immunotoxins (not yet ready for human applications), and production of human monoclonal antibodies (that could still expose the recipient to anti-idiotypic sensitization). Our results indicate that a simpler approach might be to use consecutively different antibodies with distinct idiotypes. The change in isotype might

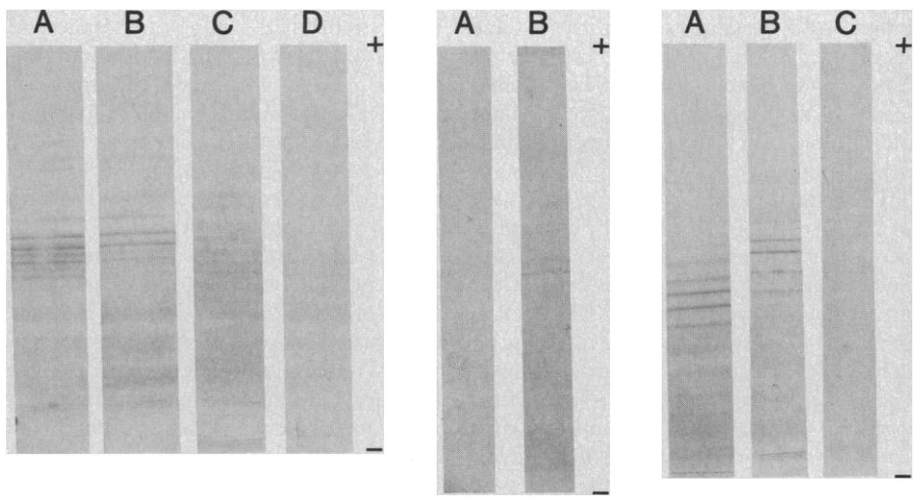

Fig. 2 (left). Serum from patient Q.U.O. collected at day 25 of OKT3 treatment and stained with (A) OKT3 and GAM, (B) Fab fragments of OKT3 and GAM, (C) OKT8 and GAM, and (D) GAM. Fig. 3 (center). Serum from patient H.A.D. collected at day 12 of OKT3 treatment stained with (A) OKT3 and GAM and (B) OKT8 and GAM. This patient, who presented anti-OKT3 antibodies with exclusive anti-isotypic specificity has been described elsewhere (*13*). Fig. 4 (right). Sera collected from monkeys treated with OKT4 monoclonal antibody (anti-helper T cells), stained with OKT4 and GAM. Monkeys in (A) and (B) showed high levels of anti-OKT4 antibodies as detected by specific ELISA. The monkey in (C) did not exhibit any anti-monoclonal response.

not be necessary since exclusive anti-isotypic immunization does not abrogate the immunosuppressive capacity of OKT3, as is illustrated by the patient described above (Fig. 3) (*12–14*).

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Methylcyclopentanoid Monoterpenes Mediate Interactions Among Insect Herbivores

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Beetles secrete an array of chemicals generally believed to mitigate attack by predators. Methylcyclopentanoid monoterpenes secreted by larvae of the willow leaf beetle, *Plagiodera versicolora*, deter feeding by conspecific adults. Furthermore, the secretion elicits a strong repugnancy response in larvae of another willow herbivore, *Nymphalis antiopa*. Leaves bearing beetle larvae are less likely than leaves not bearing beetles to be frequented and consumed by *Nymphalis* larvae. Predator defense may not be the sole function of glandular secretions produced by herbivorous insects; secretions may also mediate interactions among herbivores that use a common resource.

MANY SPECIES OF LEAF BEETLES belonging to the family Chrysomelidae have evolved exocrine glands that produce an array of chemicals (*1, 2*). Typically, nine pairs of glands line the dorsal margins of the thorax and abdomen of larvae. When disturbed, larvae evert these glands and expose drops of secretion (*3, 4*). The chemical composition of these secretions varies within and among species and is influenced by host plant chemistry in some species (*1, 2, 4*). The major classes of chemical compounds secreted by the Chryosomelinae include six types of methylcyclopentanoid monoterpenes, salicylaldehyde, benzaldehyde, juglone, and phenyl esters (*2, 4*).

The ecological role of exocrine secretions in the Chrysomelidae is generally believed to be one of defense against vertebrate and invertebrate predators (*5*). However, of the many trophic associations of leaf beetles, predators pose only one threat. Because of their habit of feeding on leaf surfaces, many Chrysomelidae larvae share their food with a variety of vertebrate and invertebrate herbivores. In addition to competing for resources, small herbivores risk the danger of being consumed by larger ones as they graze (*6*). One solution to this problem is to feed selectively on plant parts unlikely to be eaten by large consumers (*6*). An alternative is to defend the occupied resource chemically and make it unattractive to other consumers. While this latter possibility has received little attention, it is the focus of this paper.

The leaf beetle *Plagiodera versicolora* (Laich.) is an oligophagous herbivore whose larvae and adults consume the foliage of willows and poplars (*7*). The chemical compositions of glandular secretions produced by *Plagiodera* larvae are mixtures of methylcyclopentanoid monoterpenes. Compounds reported in the secretion include plagiodial, epiplagiodial, chrysomelidial, epichrysomelidial, and plagiolactone (*1, 2, 8, 9*). Analysis of the glandular secretions of beetles used in this study confirmed the presence of two methylcyclopentanoid monoterpenes similar to those found in other American beetle populations (*10*).

The genus *Salix* includes one of the largest faunas of invertebrate herbivores (*11*). In view of this rich diversity, the potential for interactions between species is great. For larvae of *Plagiodera* this situation is further complicated by adults feeding on the same plant. We tested the hypothesis that *Plagiodera* larvae can influence the feeding behavior of other herbivores and that this effect is mediated by the glandular secretion. A series of bioassays examined intraspecific interactions between *Plagiodera* larvae and adults (*12*). We first investigated the ability of beetle larvae to exclude adults from a resource. In each petri plate, five first-instar *Plagiodera* larvae were placed on one of two randomly chosen leaf disks (0.32 cm^2) punched from a single leaf. Two adult leaf beetles were added to each plate and allowed to feed. At the end of 7 hours, leaf disks bearing *Plagiodera* larvae were eaten less than disks without larvae ($T = 34$, $n = 18$, $P < 0.024$, Wilcoxon matched-pairs signed-ranks test) (Fig. 1).

We were concerned that the mere presence of larvae rather than the defensive secretion might prevent adult beetles from consuming a leaf. To clarify the role of the defensive secretion in the interaction, we conducted two bioassays. In the first, larvae were frozen to make them incapable of secreting chemicals and placed on leaf disks at a density of five larvae per disk; the remainder of the assay was conducted and evaluated as the previous one had been. The second set of assays used defensive secretions only. As before, leaf disks were placed in petri plates. One disk was treated with the secretion of two *Plagiodera* larvae expressed by pinching the caudae of the larvae and wiping the secretion on the leaf surface. Two *Plagiodera* adults were added to the plates and allowed to feed for 50 minutes. Leaf disks protected by frozen larvae or the secretion alone were consumed less than unprotected disks (dead larvae, $T = 12$, $n = 20$, $P < 0.005$); larval secretion, $T = 1$, $n = 6$, $P < 0.031$) (Fig. 1).

Plagiodera larvae were able to exclude adults of their own species from a common food resource. Furthermore, this exclusion was mediated by the glandular secretion. Leaf disks with dead beetle larvae were consumed less than those without larvae. The mode by which adult leaf beetles detect and avoid incapacitated larvae is unknown. However, defensive secretions adhere to the cuticle of Chrysomelidae (*13*). Perhaps the cuticle of dead larvae was contaminated with traces of secretion that were detected and avoided by adults.

We investigated interspecific interactions

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