suggests that the observed regenerated synapses are functional. This suggestion is further supported by the observation that a second lesion of the spinal cord just proximal to the first, in animals that had recovered the ability to swim, resulted in an immediate paralysis persisting for at least 24 days. The return of relatively normal locomotor function in the lamprey despite grossly aberrant patterns of regenerated synaptic connections supports similar findings in the goldfish (*13*). Despite the different spatial location of the regenerated junctions in the cord, the presynaptic elements may be still connecting to different regions of their normal postsynaptic target neurons.

The increase in branching may be related to the relatively short distance that regenerated neurites grow distal to the lesion. It has been hypothesized that neurons are genetically programmed to produce a relatively fixed surface area of plasma membrane (*14*). If this area is produced by profuse branching, the critical point at which growth stops may be reached closer to the lesion than if elongating growth were confined to a single neurite.

The formation of scar tissue (for example, ependymal or glial or both) has been implicated in producing a local physical barrier to the growth of nerve fibers across a lesion in the central nervous system (*15*). Scar tissue in the lamprey spinal cord appears to be composed principally of proliferating loosely aggregated ependymal cells. We found no collagen within the wound, although substantial quantities surround the normal cord (*16*). The resulting extracellular spaces between ependymal cells may provide channels for regenerating neurites to penetrate the lesion. A similar suggestion has been made for successful spinal regeneration in the newt (*17*).

We have demonstrated that axons from identified regenerating neurons grow through a spinal lesion and form new synapses caudal to the injury. This process is accompanied by functional recovery in the lamprey. The ability to follow growth and synaptic regeneration in identified reticulospinal neurons of the lamprey may permit this preparation to serve as a model for examining the cellular factors concerned with functional recovery of the injured vertebrate CNS.

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Monoclonal Antibodies Defining Distinctive Human T Cell Surface Antigens

Abstract. *Three novel monoclonal antibodies (designated OKT1, OKT3, and OKT4) were generated against surface determinants of human peripheral T cells. Both OKT1 and OKT3 reacted with all human peripheral T cells and 5 to 10 percent of thymocytes but differed in their reactivities with T cell lines. By contrast, OKT4 reacted with 55 percent of human peripheral T cells and 80 percent of thymocytes. All three antibodies were selective for T cells in the peripheral blood in that they did not react with normal B cells, null cells, monocytes, or granulocytes.*

There is now ample evidence from work with mouse and man that lymphocytes, which appear relatively homogeneous morphologically, consist of subclasses of cells with differing functions and that these subclasses have distinctive cell surface antigens that can be identified with appropriate antiserums (*1*, *1a*). In man, such analyses of T cell surface antigens have been made with spontaneous autoantibodies, with heteroantiserums, and with serendipitously discovered markers such as rosetting of sheep erythrocytes (E) and bindings of the Fc (crystallizable fragment) of immunoglobulins (*2*). These techniques have provided valuable scientific insights but have been of limited value in clinical medicine because of the heterogeneity of antibody preparations obtained, frequent low titers, and the multiple absorptions required for production of specific heteroantiserums.

The hybridoma technique of Kohler and Milstein (*3*) provides a new approach to this problem whereby the multispecific responses to complex antigens are reduced to a series of monospecific responses by cloning. A number of hybridomas secreting monoclonal antibodies to various human cell surface antigens have been described (*3*, *4*). We report our methods for preparing monoclonal antibodies to the cell surface antigens of human T cells by immunizing mice, fusing their spleen cells with a mouse myeloma

Table 1. Immunofluorescent binding data from hybridoma supernatants in two fusion experiments. Single cell suspensions from mice immunized with human peripheral E^+ cells were mixed with P3 × 63Ag8U$_1$ myeloma cells and fused with PEG as described (*3*). After the fusion, cells were washed once in a mixture of DME, 10 percent IFS, and 10 percent IHS, and were resuspended in HAT medium (*5*, *12*). In experiment 1, cells were distributed in 1-ml portions into 100 wells, and in experiment 2, into 384 wells of Costar culture plates (Costar catalog No. 3524). Seven to 10 days after fusion, 0.5 ml of HAT medium (*12*) was added to each well. Supernatant from each well showing hybridoma growth was incubated with E^+ and E^- human mononuclear cells and binding was determined by an indirect immunofluorescence assay (*7*). In each experiment supernatants from only two wells showed specific reactivities for E^+ cells and these hybridomas were studied further.

Number of wells showing growth	Binding of supernatant with	
	E^+ cells	E^- cells
	Experiment 1	
50	−	Not tested
23	+	+
2	+	−
	Experiment 2	
277	−	Not tested
32	+	+
2	+	−

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line, and cloning the resultant hybridomas. Furthermore, we emphasize the method that we used successfully to screen for antibodies with binding specificity for T cells, and describe the production of three hybridomas secreting monoclonal antibodies recognizing distinctive human T cell surface antigens with differing cellular representations.

Female mice (8 weeks old) of strains BALB/cJ or CAF_1 (Jackson Laboratory) were immunized intraperitoneally with 2×10^7 E rosette-purified peripheral T cells in PBS at 14-day intervals (*5*). Four days after the third immunization the spleens were removed and a suspension of single cells was prepared. Cell fusions were carried out according to the procedure developed by Kohler and Milstein (*3*). For each fusion, 1×10^8 splenocytes were fused in 35 percent PEG in 5 percent DMSO and RPMI 1640 with 2×10^7 P3 × 63Ag8U_1 myeloma cells (Table 1).

After cell fusion, the cells were distributed into 100 to 400 wells and cultured in selective medium (see Table 1). The wells were observed regularly under an inverted phase microscope and as cells grew up supernatants from those wells were harvested and tested for binding to human peripheral lymphocytes separated into E rosette-positive (E^+) and E rosette-negative (E^-) populations (*6*). Binding was detected both by radioimmunosassay and indirect immunofluorescence techniques (*1a*, *7*).

Table 1 summarizes the results from two experiments. In each case the majority of supernatants failed to react with E^+ cells and the contents of these wells were discarded. A fair proportion of wells in each experiment yielded supernatants that bound both E^+ and E^- cells. Although these wells may have contained antibodies to potentially interesting cell surface molecules they did not meet our criteria for the selection of T cell-specific antibodies and were not studied further. In each of two experiments two wells contained hybridomas secreting T cell-specific monoclonal antibodies. These were cloned and recloned by the limiting dilution method (*8*) to ensure that a single hybridoma was indeed secreting a monoclonal antibody with T cell specificity. That clones were formed was further suggested by the persistence of specific staining patterns in numerous subclones and the restriction of each monoclonal antibody to a single immunoglobulin subclass. The resultant clones were then given the designations OKT1 through OKT4, and larger amounts of antibody were obtained by injecting 10^7 hybridoma cells into mice primed with 0.5 ml of pristane (Aldrich) 7 days previously. The subsequent malignant ascites from these mice were then harvested and used as reagents to characterize the binding properties of the monoclonal antibodies on different human cell populations.

The results are summarized in Fig. 1 and Table 2. We found that OKT1 and OKT2, which were obtained from the same fusion, were both of the subclass immunoglobulin G_1 (IgG_1) and had identical patterns of reactivity; they probably represent daughter clones from a single parent clone in the immune spleen. The antibodies OKT1 and OKT3 had similar patterns of reactivity with peripheral T cells and thymocytes. However, OKT3 was of the IgG_2 subclass and the antibodies were also clearly distinguishable by complement-fixing properties (nega-

Table 2. Cellular representation and other characteristics of three monoclonal antibodies specific for human T cells. Antibodies OKT1 and OKT3 showed similar patterns of reactivity on peripheral cells and thymocytes but were distinguishable by their differing reactivities with T cell lines, their immunoglobulin class, and their complement-binding properties. The binding pattern of OKT4 was distinct from that of OKT1 and OKT3.

Tests	Monoclonal antibodies		
	OKT1	OKT3	OKT4
Percentage of reactivity with			
Peripheral T cells (ten samples)	> 95	> 95	55
Peripheral B cells (ten samples)	< 2	< 2	< 2
Peripheral null cells (ten samples)	< 2	< 2	< 2
Thymocytes* (eight samples)	5 to 10	5 to 10	80
Reactivity with			
B cell lines†	−	−	−
T cell lines†			
HJD-1	+	(±)	−
CEM	+	−	+
Laz 191	+	−	−
HM1	+	−	−
IgG subclass	IgG_1	IgG_2	IgG_2
Complement fixation	−	+	+
Antibody titer (fluorescence binding)	30,000	100,000	50,000

*From patients aged 2 months to 18 years. †Obtained from H. Lazarus, Sidney Farber Cancer Institute. The B cell lines Laz 256, 156, 007, and SB were obtained by Epstein-Barr virus transformation of human peripheral B cells; the T cell lines HJD-1, CEM, Laz 191, and HM1 were established from leukemic patients.

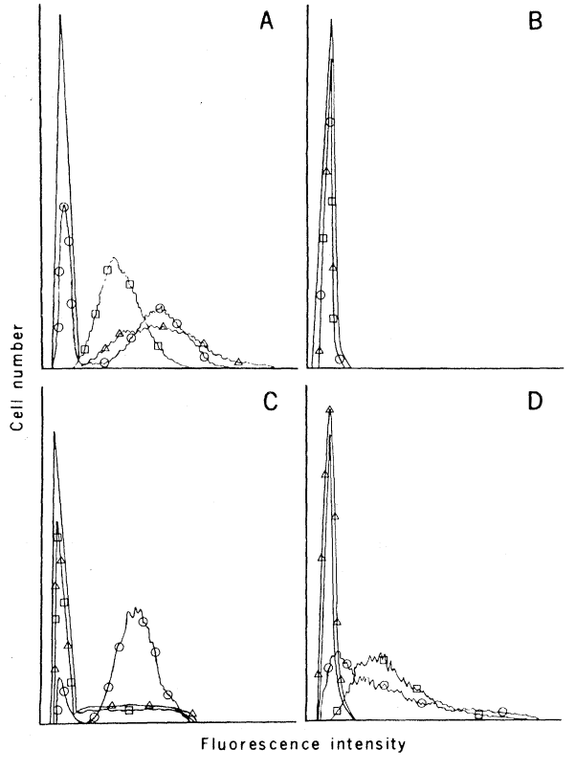

Fig. 1. Cytofluorograf (Ortho Instruments) analysis of fluorescent staining of human cells incubated with ascites from OKT1 (□), OKT3 (△), OKT4 (○), or control ascites induced with P3 × 63Ag8U_1 myeloma cells (—). (A) With E^+ peripheral blood mononuclear cells; (B) with E^- peripheral blood mononuclear cells; (C) with human thymocytes; and (D) with CEM cells, a human T cell line. Each antibody showed a distinctive pattern of staining with regard to cell distribution and fluorescent intensity.

tive for OKT1 and positive for OKT3) and by their differing patterns of reactivity with T cell lines. The binding pattern of OKT4 was quite distinct from OKT1 and OKT3; OKT4 bound only 55 percent of peripheral T cells and 80 percent of thymocytes. Furthermore, the pattern of binding of OKT4 to T cell lines was distinct from that of OKT1 and OKT3. Detailed studies demonstrated that OKT1, OKT3, and OKT4 were all nonreactive with peripheral B cells, monocytes, granulocytes, null cells, and B cell lines (*9*).

We have previously shown that the OKT1$^+$ population of peripheral lymphocytes are indeed the cells responsible for the T cell functions of mitogen reactivity, mixed lymphocyte culture responsivity, and proliferate response to soluble antigens. Furthermore we have shown that OKT1 defines a minor population of thymocytes responding in mixed lymphocyte culture but lacking mitogen responsivity (*9*). Preliminary studies suggest that OKT4$^+$ peripheral cells contain the helper T cell population.

Our present studies demonstrate the generation of monoclonal antibodies identifying antigens restricted to functional subclasses of T cells. Although our procedures entailed immunizing mice with the entire range of antigens represented on human T cells, we were able to retrieve hybrid cells producing monoclonal antibody reacting selectively with T cell subpopulations. Such antibodies should be invaluable for both diagnosis (*10*) and therapy (*11*) in clinical medicine.

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Ciliary Membrane Alterations Occurring in Experimental *Mycoplasma pneumoniae* Infection

Abstract. *Experimental infection of hamster ciliated tracheal epithelium in organ culture with virulent* Mycoplasma pneumoniae *resulted in the deterioration of ciliary necklaces and an altered distribution of membrane-associated particles on the shafts of the affected cilia. To our knowledge this is the first report of an altered disposition of ciliary membrane-associated particles in response to a specific infectious agent.*

Infections due to *Mycoplasma pneumoniae* are a major cause of respiratory disease in children and young adults (*1*). The general clinical spectrum of the disease ranges from upper respiratory tract infections, bronchitis, and tracheitis to primary atypical pneumonia (*1*). The disease is self-limiting and rarely fatal (*2*).

Investigations of the cellular basis of experimental and natural *M. pneumoniae* disease (*2–4*) have revealed ciliostasis and degenerating abnormal epithelial cells. Ultrastructural and biochemical studies also have demonstrated that in order to be fully pathogenic the organism must be specifically oriented to the host cell epithelial surface by means of a specialized terminal organelle (*4*). With the use of the freeze-etch technique, a new facet of the pathogenesis of experimental *M. pneumoniae* disease has been revealed. In these experiments, freeze-etching of hamster tracheal epithelium after incubation in organ culture with virulent *M. pneumoniae* demonstrated marked configurational alterations of the membrane-associated particles of the cilia (*5*).

Inspection of the freeze-etch replicas showed that control epithelial cells maintained in organ culture up to 48 hours retained ultrastructural integrity with their in vivo correlates (Fig. 1, A and B). A particularly prominent feature revealed by freeze-etching was the ciliary necklace, a complex of membrane-associated particles at the base of each cilium (*6*). The ciliary necklaces of epithelial cell cilia from tracheae fixed immediately after resection were comprised of generally five rows of evenly spaced particles at the base of each cilium which extended up the shaft a distance of approximately 0.2 μm (Fig. 1A). Other membrane-associated particles appeared scattered randomly and uniformly over the fractured surfaces of the cilia (Fig. 1A). This configuration of ciliary necklace and membrane-associated particles was reliably retained with minimum variability in control organ culture specimens for up to 48 hours (Fig. 1B). The membrane-associated particles of the ciliary necklaces and on the shafts of the cilia measured approximately 100 Å in diameter in both freshly fixed and organ culture specimens.

Replicas from the experimentally infected specimens showed that the highly organized ciliary necklaces were severely altered within the first 24 hours. In some cases, roughly organized remnants of the necklaces were observed; however, the general view of this area illustrated the complete disarray of the necklace particles and, in many cases, the ciliary necklace was completely absent (Fig. 1C). Membrane-associated particles observed over the ciliary shaft in infected specimens also measured approximately 100 Å in diameter and appeared clumped and unevenly distributed.

Our studies have indicated that a relatively heavy inoculum of the pathogen is necessary to produce disorganization and decreased frequency of ciliary beat in vitro. In the infected tissues, *M. pneumoniae* is observed frequently inter-

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