showed MLV particles with a weight-average diameter of 152 nm as compared to the 232 nm found in HN buffer. This represents a water loss of 67 percent in the MLV and an increase in particle density to 1.0472 g/cm^3, as compared to the 1.0154 g/cm^3 assumed for the original particles.

The particle size distribution data for the SUV particles are believed to be reasonably accurate even though no correction for the osmotic effect of the sucrose-buffer mixture was used in the measurement. The osmotic activity for the much smaller SUV particles is expected to be considerably less than that for MLV as a result of molecular constraints caused by the minimal radius of the SUV particles.

In the SFFF analytical mode, it is relatively easy to collect submilligram quantities of isolate with very high resolution. If the resolution is compromised somewhat, milligram to gram quantities can be isolated by having the instrument function as a selective filter (*3*), retaining large liposomes while allowing smaller ones to be eluted in the mobile phase. In conjunction with extrusion techniques (*7, 9*), SFFF should make possible the rapid preparation of appreciable amounts of liposomes with a polydispersity close to unity.

It thus appears that SFFF is a rapid, precise, and gentle method for the analysis of the particle size distributions of noninteracting biological colloids, as we have illustrated with liposomes. We believe that SFFF will be an equally important technique in the fractionation and size analysis of other biopolymers such as nucleic acids and cellular organelles such as ribosomes, mitochondria, and nuclei.

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5. We carried out the separations in a research SFFF instrument, using a mobile phase consisting of 0.01*M* *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid (Hepes), 0.142*M* NaCl plus 0.0055*M* NaOH, *p*H 7.45 (HN buffer), with a separating channel 57 by 2.54 by 0.0254 cm. Detection was carried out with an ultraviolet spectrophotometer operated at 240 nm (sonicated liposomes, SUV) or 350 nm (multilamellar liposomes, MLV). The SUV samples were fractionated in the sucrose-HN buffer with an initial force field of 18,000 rev/min, an exponential rotor speed decay time constant of 4.0 minutes, a flow rate of 2.0 ml/min, and an initial delay of 6 minutes. The MLV sample was fractionated in the HN buffer in the same manner except for an initial delay of 4 minutes.
6. Lipids were obtained, stored, and assayed as described by F. C. Szoka and D. Papahadjopoulos, *Proc. Natl. Acad. Sci. U.S.A.* **75**, 4194 (1978). Liposomes were composed of phosphatidylserine, phosphatidylcholine, and cholesterol in a molar ratio of 1:4:5. Small unilamellar vesicles were prepared in HN buffer at a lipid concentration of 10 μmole/ml in a bath type Sonicator at 20°C and centrifuged at 115,000*g* for 90 minutes to remove large vesicles. The supernatant containing SUV was stored under nitrogen at 4°C at a lipid concentration of 5 μmole/ml. The MLV were prepared in HN buffer at a lipid concentration of 10 μmole/ml and sequentially extruded through a 0.2-μm polycarbonate membrane to obtain a defined particle size distribution (*7*) and stored under nitrogen at 4°C at a lipid concentration of 5 μmole/ml. Negatively charged liposomes were used to minimize the possibility of aggregation during the transfer of vesicles. C. Huang, *Biochemistry* **8**, 344 (1969); ——— and J. P. Charlton, *J. Biol. Chem.* **246**, 2555 (1971); S. M. Johnson, *Biochim. Biophys. Acta* **397**, 27 (1973).
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8. Since MLV are osmotically sensitive structures [A. D. Bangham, J. de Gier, G. D. Greville, *Chem. Phys. Lipids* **1**, 225 (1976)], placing them in 1.34*M* sucrose-HN buffer would cause them to shrink. This shrinkage would result in an apparently greater particle density as compared to the true density when the MLV were placed in HN buffer and determined by this method. If the density of the particles is assumed to remain constant, 1.0154 g/cm^3, the weight-average diameter in the two buffers can be calculated from retention data. The results indicate that a significant change in liposome density occurs from the loss of water when one shifts from the HN buffer to the sucrose-HN buffer.
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Human T Cell Antigen Expression by Primate T Cells

Abstract. *Phylogenetic study in primates of the reactivity of 11 monoclonal antibodies to human T cells and rosette formation in the reaction of primate T cells with sheep erythrocytes demonstrate that the sheep erythrocyte receptor and the determinants of T cell subset antigens are highly conserved during primate evolution. Other T cell antigen determinants are less well conserved. Human, gorilla, and chimpanzee T cells showed identical reactivity with the monoclonal antibodies to human T cells. The expression of human T cell antigen determinants by primate T cells suggests a constant rate of evolution for this group of molecules and provides additional evidence that man and African apes shared a relatively recent common ancestor.*

Comparisons of proteins from humans and other primates for variations in amino acid sequences, DNA restriction endonuclease cleavage patterns, and immunological cross-reactivity have provided biochemical data in support of a relatively recent common ancestor (5 million years ago) of man and African apes (*1–3*). Data have come from studies of primate albumin, transferrin, and α and β globin proteins (*1*). Recent fossil evidence, although controversial, also supports this notion (*4*). Studies have been done on the reactivity of determinants of human histocompatibility antigens A and B with lymphocytes of nonhuman primates (*5*) and on the use of monoclonal antibodies to T cells as therapeutic reagents in primates (*6*), but few phylogenetic studies have been performed to evaluate the evolution of human lymphocyte-related cell surface proteins. Hybrid cell methodology has provided the reagents needed to define the repertoire of lymphocyte surface antigens. In particular a large number of monoclonal antibodies to human antigens specific to T cells have been produced (*7–13*); the specificity and classification of these reagents have been reviewed (*14*).

Highly specific monoclonal antibodies react with only one antigenic determinant on a given molecule (*15*), and we have used a large panel of these reagents to study the expression of various human T cell antigen determinants on the T cells of nonhuman primates. Our goal was to seek evolutionary patterns of proteins specific to the immune system. Blood was drawn from one to five animals of each of the following primates: gorillas (*Gorilla gorilla*), chimpanzees (*Pan troglodytes*), gibbons (*Hylobates lar*), Old World monkeys (rhesus, *Macaca mulatta*; pig-tailed, *Macaca nemistrina*; and stump-tailed, *Macaca speciosa*), and New World monkeys (Cebus, *Cebus atella*, and spider, *Ateles fusciceps*). Mononuclear cell preparations were made on Hypaque-Ficoll density gradients. We used two methods to determine the reactivity of the monoclonal reagents with primate T cells; indirect immunoflu-

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Table 1. Reactivity of peripheral blood T cells from various primates with monoclonal antibodies to human T cells. Monoclonal antibodies L17F12, T101, 10.2, DU-SKW3-1, 9.6, OKT3, A-50, and 3A1 bind to most human peripheral T cells, whereas OKT4, OKT8, and 9.3 bind to subsets of human peripheral T cells (*14*). Values are expressed as the percentage ± standard error of T cells showing reactivity with the monoclonal antibody.

Subject*	Monoclonal antibody									
	L17F12	T101	10.2	DU-SKW3-1	9.6	OKT3	A-50	3A1	OKT4	OKT8	9.3
Humans	97	99	98	98	100	95	95	85	65	30	64
Gorillas	89 ± 3	90 ± 6	98 ± 6	97 ± 3	99 ± 1	91 ± 7	84 ± 12	97 ± 2	53 ± 6	46 ± 9	89 ± 11
Chimpanzees	83 ± 2	68 ± 8	64 ± 1	81 ± 2	96 ± 4	78 ± 9	59 ± 7	90 ± 4	51 ± 3	72 ± 6	76 ± 8
Orangutan†	100 ± 0	99 ± 1	84 ± 16	95 ± 5	100 ± 0	0 ± 0	91 ± 9	94 ± 6	42 ± 22	92 ± 4	80
Gibbons	86 ± 10	78 ± 15	N.D.‡	86 ± 14	100 ± 0	99 ± 1	0 ± 0	11 ± 3	19 ± 7	87 ± 11	65 ± 20
Rhesus monkeys	0 ± 0	0 ± 0	0 ± 0	63 ± 19	100 ± 0	0 ± 0	0 ± 0	0 ± 0	62 ± 7	42 ± 12	N.D.
Pig-tailed monkeys	0 ± 0	0 ± 0	0 ± 0	63 ± 1	100 ± 0	0 ± 0	0 ± 0	6 ± 1	59 ± 1	48 ± 3	59 ± 5
Stump-tailed monkeys	0 ± 0	10 ± 2	0 ± 0	41 ± 5	100 ± 0	0 ± 0	0 ± 0	0 ± 0	35 ± 3	60 ± 12	50 ± 9
Cebus monkeys	0 ± 0	4 ± 3	0 ± 0	20 ± 5	N.D.‡§	0 ± 0	6 ± 4	0 ± 0	0 ± 0	10 ± 3	47 ± 20
Spider monkey‖	0	0	0	0	87	0	0	0	0	0	41

*Three to five animals were studied in each case, except where noted. As controls, lymphocytes from two rabbits were also studied; rabbit lymphocytes did not react with any of the monoclonal antibodies. Data on human T cells represent the means for six normal subjects (*17*). †Two orangutans were studied for each T cell antibody except 9.3, for which only one was studied. ‡Not done. §But 91 ± 5 percent of the Cebus monkey lymphocytes rosetted with sheep erythrocytes. ‖One spider monkey was studied.

orescence and microcytotoxicity (*16, 17*). In each assay, background fluorescence was determined by incubation of cells with P3 × 63/Ag8 ascitic fluid (control murine myeloma protein) and fluorescein-conjugated antibodies to mouse immunoglobulin G. In every instance less than 5 percent of the cells were nonspecifically fluorescent.

All of the primate T lymphocytes that we tested rosetted with neuraminidase-treated sheep erythrocytes in percentages equivalent to those of human T cells (81 ± 2 percent of lymphocytes). The reactivity of gorilla and chimpanzee T cells with our panel of reagents was identical to that of humans (Table 1). Orangutan cells were reactive with 10 of the 11 reagents (lacking only the OKT3 antigen), and gibbon cells reacted with 9 of the 11 reagents (lacking 3A1 and A-50 antigens). In contrast, cells of all Old World monkey species tested were similar and reacted with only 5 of the 11 human T cell–specific reagents. Antibodies L17F12, T101, 10.2, OKT3, A-50, and 3A1 (antibodies reacting with a majority of human peripheral T cells) did not react with Old World monkey cells. The Old World monkey T cells, in addition to having an E-rosette receptor, reacted with monoclonal antibodies OKT4, OKT8, and 9.3 (antibodies defining functional subsets of human peripheral T cells) (*8, 18*); these cells also uniformly reacted with antibody DU-SKW3-1, a murine monoclonal antibody that precipitates a 65,000-dalton molecule similar to the molecule detected by reagents L17F12, T101, and 10.2 [the human analog of the murine Lyt 1 molecule (*19*)]. Either DU-SKW3-1 binds to a different determinant than L17F12, T101, and 10.2 do, or it binds to a different 65,000-dalton molecule (*20*).

Cells from New World monkeys (spider and Cebus monkeys) reacted only with the E-rosette-receptor antibody (9.6) and the antibody to the inducer T cell (9.3). Cebus monkey cells also reacted with the pan–T cell antibody DU-SKW3-1 (*21*).

The study of fossil and biochemical data (*1–4*) suggest a common ancestor for chimpanzees, gorillas, and man about 5 million years ago. In contrast, the common ancestor for man, African apes, and orangutans is estimated at 8 million years, and for man, African apes, orangutans, and gibbons at 10 million years. The divergence of Old World monkeys and the higher primates is judged to have occurred 30 million years ago. Although it is generally agreed that New World monkeys constitute a lineage separate from that of Old World monkeys, African apes, and man, the time of a common ancestor is difficult to establish because of the lack of fossil data (*22*).

Plotting the number of T cell antigens absent on T cells of the various primate species against the estimated time of evolutionary divergence demonstrated a correlation (R^2 = .99), with a constant apparent rate of evolution for the T cell antigens as a group (slope, 4.17) (Fig. 1) (*23*).

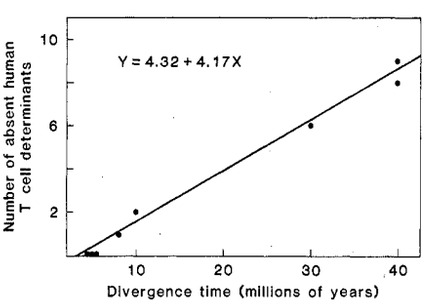

Fig. 1. The number of determinants of human T cell antigens that are not detected on the T cells of a particular primate species plotted against the estimated time of divergence for primate species (*2*).

These data provide additional molecular evidence for a relatively recent common ancestor for man, chimpanzees, and gorillas, and support the existing proposed ranking of evolutional divergence of hominids. Moreover, the high degree of conservation of T cell subset antigen determinants suggests that these molecules may have been important in conferring a selective evolutionary advantage (*24*).

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16. Blood, drawn from six normal subjects, was separated by Hypaque-Ficoll density centrifugation. T cells were identified and isolated by sheep erythrocyte rosette formation and density centrifugation. Data in Table 1 on the reactivity of monoclonal antibodies with T cells were determined by an indirect fluorescent assay. In addition to testing primate cells for T cell–specific reagents, we determined reactivity with antibodies that define nonpolymorphic determinants of the human histocompatibility antigen, A, B, and C molecule (3F10) and an Ia-like molecule (L-243). Cells from all the primates we tested reacted with antibody to human histocompatibility antigens, and a subset of all primate peripheral blood cells was positive for the Ia-like antigen. All primate T cells formed rosettes with sheep erythrocytes (81 ± 2 percent of lymphocytes)
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20. Antibodies L17F12, T101, and 10.2 may bind to the same antigenic determinant on the 65,000-dalton human Lyt 1 analog [R. I. Fox, P. Kung, R. Levy, in *Lymphocyte Differentiation*, N. Warner, Ed. (Academic Press, New York, in press)]. In Table 1, each monoclonal antibody to T cells is reported separately and considered to bind to a distinct determinant. Monoclonal antibody DU-SKW3-1 was raised against the T cell chronic lymphocytic leukemia cell line SKW-3. Analysis by sodium dodecyl sulfate–polyacrylamide gel electrophoresis has not resolved whether the 65,000-dalton molecule detected by DU-SKW3-1 is the same as that recognized by antibodies L17F12, T101, and 10.2 (unpublished data).
21. While 65 percent of human peripheral T cells are positive for OKT4 and 30 percent are positive for OKT8, it was not uncommon to see reversal of this pattern of T cell subset antigens. Thus in gibbons only 19 ± 7 percent of the T cells were positive for OKT4 and 87 ± 11 percent were positive for OKT8. Moreover, in orangutans and chimpanzees, significant proportions of peripheral T cells expressed both the OKT4 and OKT8 antigens (Table 1).
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23. The evolutionary divergence date for New World monkeys is unknown, but plotting the number of absent human T cell antigen determinants yields an apparent divergence time of 40 million years ago for New World monkeys. This time may be arbitrary since the total number of human T cell antigens is not known. Moreover, an assumption made in the interpretation of data in Fig. 1 is that it is valid to consider the T cell antigens as a group in estimating evolutionary divergence.
24. Supported by NIH grants CA28936, CA08975, CA11265, and CA11525. B.L.D. is a special fellow of the Leukemia Society of America. We thank J. Lowery for assistance with the manuscript, and A. McMichael, R. Levy, E. Engleman, I. Royston, J. Hansen, G. Eisenbarth, G. Goldstein, E. Reinherz, L. Boumsell, and A. Bernard for exchange of reagents.

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Developmental Equations for Postural Tremor

Abstract. *Developmental equations for the electroencephalogram have been reported previously for normal children and brain disorder has been shown to cause substantial deviations from this developmental norm. The present report describes directly parallel phenomena with regard to postural tremor.*

Developmental equations that describe electroencephalographic (EEG) changes in children aged 6 to 16 years have been reported by John *et al* (*1*). These authors noted that the regression coefficients associated with the β, α, θ, and δ frequency bands taken from the parieto-occipital, central, temporal, and frontotemporal regions of the brain change systematically with age. Data based on 306 U.S. children agreed well with data collected on 342 Swedish children. A third group of 91 normal children were reported to be quite similar in their development to the other two groups of normal children (*2*). Substantial deviations from this normal developmental sequence were then reported for 474 children "at neurological risk," 143 learning disabled children, and 163 specific learning disabled children (*2*). Normal development is associated with an increase in the dominant EEG frequency; brain damage or deterioration is associated with a lowering of this dominant frequency (*1*).

The purpose of the study reported here was to identify parallel developmental and pathological changes related to postural tremor. The developmental equations stem from a reanalysis of previously published data on 287 children aged 2 to 16 years (*3*). The data on the effects of brain pathology on postural tremor come from two previously published studies on adults (*4, 5*).

Marshall (*3*) asked 287 normal children, ranging in age from 2 to 16 years, to hold an accelerometer (weighing 65 g and measuring 9.5 cm in length and 3.3 cm in diameter) in the palm of their outstretched hand for a brief but unspecified time. Although the subjects were instructed or coached to hold their arm as steady as possible, small movements, described as physiological tremor, remained. Marshall calculated the dominant or peak frequency for each child and displayed these data in a figure relating tremor frequency to the subject's age. Upon reanalyzing Marshall's data, I discovered an unreported correlation [$r(13) = .9433, P < .001$] between average age and average peak frequency. This result clearly establishes an average developmental sequence that is well described by Eq. 1.

$$Y = .2612X + 4.4417 \quad (1)$$

Table 1 shows the average peak frequency and standard deviation associated with each age group studied by Marshall plus the value predicted for that age group by Eq. 1. The difference between the observed and predicted peak frequency values is also reported. Both the obtained data and the theoretical calculations show that the peak frequency slowly and regularly increases as a function of chronological age. The slope of Eq. 1 reveals that the peak frequency increases by 0.2612 Hz or about 0.25 Hz per year. This developmental sequence continues until the adult frequency of 10 Hz is reached. Equation 1 predicts that the 10-Hz peak frequency is reached

Table 1. A statistical analysis of data reported by Marshall (*3*) on the peak frequency of physiological tremor in 287 normal children (in hertz).

N	Age (years)	Average peak frequency	Standard deviation of peak frequency	Predicted peak frequency	Deviation from prediction
3	2	5.33	5.77	4.96	0.37
4	3	5.75	0.50	5.23	0.52
10	4	5.80	0.79	5.49	0.31
20	5	5.60	1.05	5.75	−0.15
14	6	6.00	1.04	6.01	−0.01
18	7	5.78	1.06	6.27	−0.49
20	8	5.90	1.21	6.53	−0.63
22	9	6.23	1.41	6.79	−0.56
25	10	7.28	1.93	7.05	0.23
34	11	7.06	1.74	7.32	−0.26
30	12	7.60	1.73	7.58	0.02
22	13	7.45	1.68	7.84	−0.39
30	14	8.23	1.61	8.10	0.13
22	15	8.50	1.50	8.36	0.14
13	16	9.38	1.61	8.62	0.76

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