types were shown to differ in their temperature stabilities. Leukocyte IFN was the most stable at 56 °C, whereas immune IFN was the least stable. Fibroblast IFN was intermediate. Instability of bovine fibroblast IFN has also been reported previously[17]. Differences were also noted regarding stability at 45 °C where fibroblast IFN lost 40% of its original titer, whereas leukocyte was completely stable. Because of the lack of specific antibodies to these IFNs, it is difficult to adequately characterize and separate the various classes.

A number of reports have described cross-species activities between mammalian IFNs[18–21]. In the present study both bovine leukocyte and fibroblast IFNs were tested for their ability to protect heterologous cells. Results indicated that bovine fibroblast IFN was more effective in protecting heterologous cells than leukocyte IFN. This was in contrast to results reported by Carter et al.[24] using porcine IFN. It is possible that this could relate to the extent of glycosylation, and/or purity, however, data in this area are not available.

1 Supported in part by National Institutes of Health Biomedical Research Support Grant RR05773-08.
2 Address reprint requests to Dr Stanley S. Lefkowitz.
3 Lefkowitz, S.S., and Luna, V.E.R., Intervirology *21* (1984) 221.
4 Lefkowitz, S.S., Talley, R.S., and Lefkowitz, D., Clin. Immun. News *19* (1980) 1.
5 Babiuk, L.A., and Rouse, B.T., Infect. Immun. *136* (1976) 1567.
6 Johnson, H.M., Stanton, G.J., and Baron, S., Proc. Soc. exp. Biol. Med. *154* (1977) 138.
7 Epstein, L.B., Tex. Rep. biol. Med. *35* (1977) 42.
8 Wheelock, E.F., Science *149* (1965) 310.
9 Maehara, N., and Ho, M., Infect. Immun. *15* (1977) 78.
10 Youngner, J.S., and Salvin, S.B., J. Immun. *111* (1973) 1914.
11 Ahl, R., and Rump, A., Infect. Immun. *14* (1976) 603.
12 Rosenquist, B.D., and Loan, R.W., Am. J. vet. Res. *28* (1967) 619.
13 Rosenquist, B.D., and Loan, R.S., Am. J. vet. Res. *30* (1969) 1293.
14 Fulton, R.W., and Rosenquist, B.D., Am. J. vet. Res. *37* (1976) 1497.
15 Cummins, J.M., and Rosenquist, B.D., Am. J. vet. Res. *41* (1980) 161.
16 Cummins, J.M., and Rosenquist, B.D., Am. J. vet. Res. *43* (1982) 955.
17 Fulton, R.W., and Pearson, N.J., Can. J. comp. Med. *46* (1982) 100.
18 Jankowski, W.J., Davey, M.W., O'Malley, J.A., Sulkowski, E., and Carter, W.A., J. Virol. *16* (1975) 1124.
19 Davey, M.W., Sulkowski, E., and Carter, W.A., Biochemistry *15* (1976) 704.
20 Babiuk, L.A., and Rouse, B.T., Intervirology *8* (1977) 250.
21 Carter, W.A., Davis, L.R., Jr, and Chadha, K.C., Molec. Pharmac. *15* (1979) 685.

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T cell and T cell subset determination in normal peripheral blood: comparison of the indirect immunofluorescence and lymphocytotoxicity techniques

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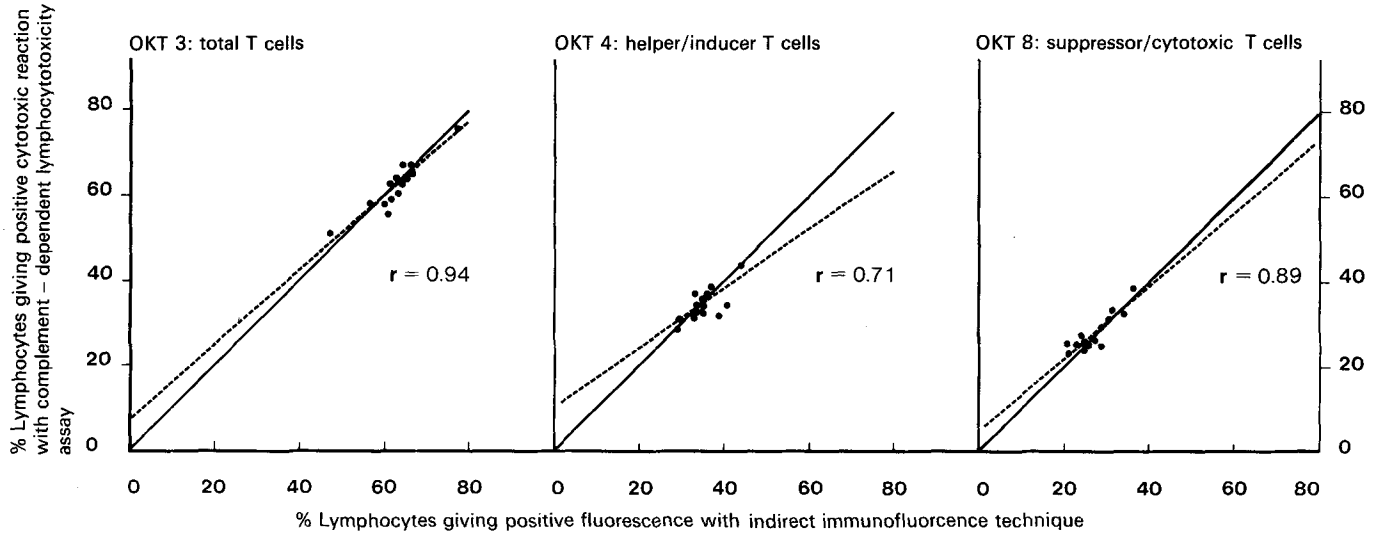
Summary. We compared a simple complement-dependent lymphocytotoxicity test with a widely used indirect immunofluorescence procedure to enumerate total T, T helper, and T suppressor lymphocytes in normal blood samples. Results with the two techniques were closely similar.
Key words. Blood, peripheral; T cells; immunofluorescence technique; lymphocytotoxicity technique.

Perturbations in immunoregulatory T lymphocyte dynamics have been observed in a variety of immunopathologic disorders[1], and determination of circulating T helper and T suppressor cells has become a common procedure in both clinical and research laboratories. T cell subset analysis has been most commonly performed by indirect immunofluorescence, using either manual or cytofluorographic techniques[2,3]. However, a simple manual technique which requires only a standard laboratory microscope for the visualization and enumeration of T helper and T suppressor cells would be an advantage for a small laboratory. In principle, it should be possible to use a lymphocytotoxicity test for T cell enumeration since the monoclonal anti-T cell antisera fix complement[4].

Materials and methods. We studied total T, T helper, and T suppressor cells in 14 healthy subjects (9 males, 5 females; age 27–45 years) using complement-dependent lymphocytotoxicity and the standard manual indirect immunofluorescence techniques in parallel.

Peripheral blood mononuclear cells were isolated by ficoll-hypaque density gradient centrifugation[5]. We used murine monoclonal antisera termed OKT3, OKT4, and OKT8 to characterize all T, T helper, and T suppressor cells respectively. Lymphocytotoxicity was determined by trypan blue exclusion using rabbit serum as the source of complement. 80 µl of mononuclear cell suspension (concentration 2.5×10^6 cells/ml) and 10 µl of appropriately diluted OKT3, OKT4 and OKT8 antibody, and phosphate-buffered saline (control) were placed in 12 × 75 mm round bottom glass test tubes and incubated for 5 min at 37 °C. 10 µl of rabbit serum was added to each test tube which was then further incubated for 45 min. Thereafter, 100 µl of 0.2% trypan blue solution were added and 10 min later, 1–2 drops of the cell suspension were placed in a Neubauer hemocytometer. The number of living and dead cells were counted using a standard light microscope equipped with a 40 × objective. Approximately 200 cells were counted and all determinations were performed in duplicate. Lymphocytotoxicity was expressed as the percentage of cells killed by each antiserum. The indirect immunofluorescence technique was performed according to the procedure recommended by the serum supplier (Ortho Pharmaceutical, Raritan, N.J.). Briefly, aliquots of 1×10^6 mononuclear cells in 200 µl of wash medium (RMPI-1640, 5% fetal calf serum, 25 mM hepes, Grand Island Biological, Santa Clara, Ca) were pelleted in four separate 12 × 75 mm round bottom glass test tubes, and resuspended in 5 µl of reconstituted monoclonal antibody solution, and phosphate-buffered saline (control), respectively. After 30 min incubation on ice followed by two washes with wash medium, the cells were incubated for 30 min on ice with a 1:20 dilution of a fluorescein-conjugated goat antimouse immunoglobulin (Meloy Laboratories, Springfield, Va). The cells were



Comparison of the percentage of total T, T helper, and T suppressor cells in peripheral blood of 14 normal subjects using indirect immunofluorescence and complement-dependent lymphocytotoxicity techniques. The solid line is the identity line. The broken line is the best-fitted linear regression line.

Distribution of T lymphocytes and T lymphocyte subsets in normal peripheral blood as determined by the indirect immunofluorescence and the complement-dependent lymphocytotoxicity techniques

Subject	Sex	Age	Antiserum: OKT3		OKT4		OKT8		OKT4/OKT8	
			IIF* (%)	CDLC** (%)	IIF (%)	CDLC (%)	IIF (%)	CDLC (%)	IIF	CDLC
1	M	33	60.89	56.94	36.70	38.55	25.13	25.84	1.46	1.50
2	F	27	56.67	57.94	32.51	32.29	24.61	25.29	1.32	1.28
3	F	31	47.48	51.15	40.50	34.23	20.45	25.11	1.98	1.36
4	M	39	63.21	60.26	38.69	31.88	30.95	31.25	1.25	1.02
5	M	32	65.88	65.35	43.62	43.65	36.46	39.11	1.20	1.12
6	F	28	65.38	67.11	29.63	30.27	29.17	24.90	1.02	1.22
7	M	38	77.03	75.35	33.15	37.99	28.89	29.40	1.15	1.29
8	M	31	65.89	66.74	28.65	28.42	33.68	32.45	0.85	0.88
9	F	28	61.84	59.43	33.88	34.13	22.61	25.96	1.50	1.31
10	F	43	62.39	62.56	34.62	34.66	25.11	25.19	1.38	1.38
11	M	40	65.55	65.97	36.45	36.87	24.29	26.72	1.50	1.38
12	M	41	63.73	62.35	34.41	32.70	21.34	22.77	1.61	1.44
13	M	30	60.10	58.07	34.54	35.07	26.86	26.16	1.29	1.34
14	M	44	62.81	64.41	32.95	33.28	31.79	33.40	1.04	1.00
Mean ± SEM			62.78 ± 1.69	62.40 ± 1.56	35.02 ± 1.07	34.59 ± 1.03	27.24 ± 1.27	28.11 ± 1.19	1.33 ± 0.08	1.25 ± 0.05

* IIF = indirect immunofluorescence technique. ** CDLC = complement-dependent lymphocytotoxicity assay. For IIF, % fluorescent cells in control reagent = 1.42. For CDLC, % cytotoxicity in control reagent = 5.54.

then washed twice with wash medium and resuspended in 1–2 drops of mounting medium (phosphate-buffered saline, pH 7.2 with 30% (v/v) glycerol). One drop of cell suspension was placed on a microscope slide and the slide was examined by fluorescence microscopy. Approximately 200 cells were counted per slide. For each blood sample, the percentage of total T, T helper, and T suppressor cells were calculated, based on the number of cells giving positive fluorescence with antiserum OKT3, OKT4, and OKT8 respectively.

Results and comments. Our results are shown in the table and the figure. The mean counts of total T, T helper, and T suppressor cells as well as the ratio of helper to suppressor T cells are closely similar for the two techniques (immunofluorescence vs lymphocytotoxicity: $p = 0.5$ for cell counts; $p = 0.3$ for helper:suppressor ratio). The figure shows the degree of correlation between the two techniques when the results of cell counts in each of the 14 subjects are plotted graphically. For each OKT antiserum, results of lymphocytotoxicity test correlated closely with those obtained using the immunofluorescence technique ($r = 0.94$, 0.71, and 0.89 for OKT3, OKT4, and OKT8, respectively).

Since our study includes only normal subjects, the sensitivity of the lymphocytotoxicity test at low cell counts has not been tested. However, in the first case of acquired immunodeficiency syndrome seen recently in our hospital, and in two patients with active lupus erythematosus, results with the two techniques again showed a close correlation. Our results indicated that the lymphocytotoxicity test investigated in this study is comparable in sensitivity to the method using indirect immunofluorescence. Furthermore, because of the simplicity of the procedure as well as the shorter time required for its performance, the lymphocytotoxicity test should be valuable as a simple screening method of the determination of T cell subsets instead of indirect immunofluorescence.

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1 Bach, M.-A., and Bach, J.-F., Clin. exp. Immun. *45* (1981) 449.
2 Sridama, V., Pacini, F., Yang, S.-L., Moawad, A., Reilly, M., and DeGroot, L.J., N. Engl. J. Med. *307* (1982) 352.
3 Reinherz, E.L., Kung, P.C., Goldstein, G., and Schlossman, S.F., Proc. natl Acad. Sci. USA *76* (1979) 4061.
4 Van Wauwe, J., and Goosens, J., Immunology *42* (1981) 157.
5 Boyum, A., Scand. J. clin. Lab. Invest. *21*, suppl. 97 (1968) 1.

0014-4754/84/121412-02$1.50 + 0.20/0