Normal ranges of some select lymphocyte sub-populations in peripheral blood of normal healthy Indians

R. K. Saxena[1,*], V. Choudhry[1], I. Nath[2], S. N. Das[2], R. S. Paranjape[3], G. Babu[4], S. Ramlingam[4], D. Mohanty[5], H. Vohra[6], M. Thomas[7], Q. B. Saxena[8] and N. K. Ganguly[8]

[1]School of Life Sciences, Jawaharlal Nehru University, New Delhi 110 067, India
[2]Department of Biotechnology, AIIMS, New Delhi 110 002, India
[3]National AIDS Research Institute, Bhosri, Pune 411 026, India
[4]Department of Virology, CMC, Vellore 632 004, India
[5]Institute of Immunohaematology, 13th Floor, NMS Building, KEM Hospital, Mumbai 400 012, India
[6]Department of Experimental Medicine and Biotechnology Post-Gradate Institute of Medical Education and Research, Chandigarh 160 012, India
[7]Institute for Research in Medical Statistics, Indian Council of Medical Research, New Delhi 110 029, India
[8]Indian Council of Medical Research, New Delhi 110 029, India

A national task force was constituted by the Indian Council of Medical Research (ICMR), to define reference ranges for several lymphocyte sub-populations in healthy Indians. The task force comprised six centres in different locations in India and analysed by flow cytometry, CD3, CD4, CD8, CD19, CD16 and CD56 populations in peripheral blood samples from a total of 1027 healthy Indians. National means of percentages of different sub-populations of lymphocytes were determined as follows: 68.65% (CD3); 37.10% (CD4); 34.04% (CD8); 14.67% (CD19); 14.58% (CD16) and 12.44% (CD56). Mean CD4/CD8 ratio was 1.2 for all samples. Significant geographical differences were found in percentages of CD4 and CD8 sub-populations and consequently in their ratios. In southern states, especially Tamil Nadu and Kerala, CD4/CD8 ratios were significantly lower than in northern and western parts of India. Detailed statistical analysis of the data and effects of variables like age, sex, smoking, consumption of alcohol, nutritional status, and self-perception of state of health on lymphocyte sub-populations, have been presented.

IMMUNE system sustains a healthy state by eliminating harmful microbes that intrude in the body and by maintaining an active anti-tumour surveillance system to counter spontaneous cellular transformation events. Alteration in the functioning of the immune system may therefore have a crucial bearing on the health status itself. Assessment of the immune function has become an important diagnostic and prognostic tool in the hand of modern medicine. Leukocytes and soluble components like antibodies and complements mediate the effectors phase of the immune system. Lymphocytes participating in generating an immune response belong to several distinct classes including T and B lymphocytes and the natural killer (NK) cells. Different types of lymphocytes have specific functions and are characterized by the expression on their cell surfaces certain defined CD (cluster of differentiation) markers[1]. Enumeration of different types of lymphocytes in blood circulation is generally done by the technique of phenotyping in which peripheral blood lymphocytes are stained with a panel of fluorescence-tagged monoclonal antibodies recognizing different specific CD markers, followed by flow cytometry. While in Western countries, physicians routinely use phenotyping to assess the immune status of patients, in India the procedure is as yet restricted only to larger hospitals and research institutions, because of its high cost. In future however, the use of this powerful diagnostic and prognostic technique is expected to spread rapidly.

Reference ranges of different types of lymphocytes in peripheral blood of healthy persons have been well laid out for Western countries[2–5]. Several reports about reference ranges of blood lymphocytes for countries outside the western hemisphere are also available[6–9]. Ranges of lymphocyte sub-populations in Indians have been reported, but the information is based upon relatively small number of blood samples generally confined to certain geographical areas of the country[10]. Since authentic information regarding the lymphocyte sub-populations in Indian subjects is not available, ranges of lymphocyte sub-populations established for the Western countries are currently being used in India. The need for laying out these ranges for healthy Indians had long been felt in the country. Emergence of HIV–AIDS as looming epidemics in India imparted further urgency to this task, as enumeration of T-helper lymphocytes (CD4 lymphocytes) constitutes a crucial diagnostic and prognostic test for monitoring HIV-infected patients[11,12].

In view of the urgent need to lay down the ranges of normal lymphocytes in healthy Indians, a National Task Force was constituted by ICMR research to define the reference ranges of different lymphocyte sub-populations, and various factors that can influence these values. Results of this Task Force study based upon data for a total of 1027 blood samples, have been presented in this communication.

The ICMR task force comprised of six institutions in India, which were JNU (School of Life Sciences, Jawaharlal Nehru University, New Delhi); AIIMS (Department of Biotechnology, All India Institute of Medical Sciences, New Delhi); NARI (National AIDS Research Institute, Pune); CMC (Department of Virology, Christian Medical College, Vellore); IIH (Institute of Immunohaematology, Mumbai); PGI (Department of Experimental Medicine and Biotechnology, Post Graduate Institute of Medical Education and Research, Chandigarh). Among

*For correspondence. (e-mail: rksaxena@mail.jnu.ac.in)

these institutes, JNU and IIH used Coulter Epics XL flow cytometer, whereas the rest of the institutions had Becton Dickinson FACScan flow cytometer. Each centre was assigned the job of identifying about 200 healthy individuals willing to donate blood samples for flow cytometric analysis of lymphocyte sub-populations. In each centre, all prospective donors were asked to fill up a questionnaire to obtain the basic personal information as well as detailed information about the health status and habits about the donor. The following information was collected about each blood donor: name of donor; place of origin (name the State); State of residence (for the past 5 years); sex (M/F); age (years); height (cm); weight (kg); marital status; present occupation; smoking habit (none/occasional/habitual); alcohol consumption (none/occasional/habitual); general health (personal rating, perfect/good/OK/not good); nutritional status (personal rating, perfect/good/OK/not good).

The following exclusion criteria were used: any sickness within the past one month (including viral infection, accident, trauma, etc.); any chronic (including autoimmune) diseases; history of infectious/communicable diseases (including gastro-intestinal infection within the last the 6 months); any major surgery (within the last 6 months); allergy to drugs/or any other substance; pregnancy/lactation (within the last 6 months); vaccination (within the last 6 months); blood transfusion (with the last 3 months); active drug abuse.

Different centres procured and used the monoclonal antibodies and other reagents recommended by the respective manufacturers of their flow cytometers. Protocols used for blood collection and staining of blood cells were as recommended by the suppliers of the reagents. Quality-control procedures, calibration by standard beads and colour compensation procedures were strictly done according to the instructions of the manufacturers of the flow cytometric equipment. Lymphocyte populations were gated using the forward-side scatter plot and the following specific populations were analysed: $CD3^+$ (total T-cells); $CD3^+CD4^+$ (T-helper cell population); $CD3^+CD8^+$ (T-cytotoxic/suppressor population); $CD3^-CD19^+$ (B-lymphocyte population); $CD3^-CD16^+$ (NK-cell marker); $CD3^-CD56^+$ (NK-cell marker).

Proportions of various lymphocyte sub-populations were determined from flow cytometry two-colour histograms. Values for the mean and standard deviation of each lymphocyte sub-population were generated using a Monte Carlo procedure for non-Gaussian distributions and the best-fit distribution.

Blood samples from a total of 1027 healthy Indian donors were analysed for CD3, CD4, CD8, CD19, CD16 and CD56 positive lymphocytes. The study was carried out in six centres in different parts of India. Combined data from all centres are summarized in Table 1. Mean values of percentage of lymphocytes bearing different markers along with standard error and standard deviation values and median values have been shown for each sub-population of lymphocytes (Table 1). Table 1 also shows three types of ranges of spread of datapoints for each lymphocyte population. These are (a) actual range within which 100% of the values fall, (b) range ignoring lowest and highest 5% datapoints, i.e. range of 5th and 95th percentile points, and (c) 95% confidence limit of the dataset. If the standard deviation (SD) as percentage of mean is taken as a measure of variations within datasets, CD3 data show the least variation (SD = 12% of mean). CD4, CD8 and CD19 data show intermediate variations (SD = 20 to 30% of mean), and CD16/CD56 data show the maximum variations (SD = 60 to 70% of mean).

While Table 1 shows detailed statistics of complete data collected in all six centres in India, a comparison of the statistics from individual centres that participated in this study, is shown in Table 2. These results show that for B-cell (CD19 population) and T-cell populations (CD3), the mean values from individual centres were relatively close to national averages. Variations among mean values for other lymphocyte populations were relatively greater. Since these variations could be related to the geographic location of the centre/donors, this variable was analysed in detail.

Table 1. Summarized statistics of all data on lymphocyte sub-populations

Lymphocyte sub-population	Number of samples	Mean	Standard error (SE)	Standard deviation (SD)	Median	Normal range		
						Data range[a]	5th to 95th percentile[b]	Population range[c]
CD3	1126	68.65	0.25	8.24	69.10	30.6–96.0	53.0–80.0	68.2–69.2
CD4	1126	37.10	0.23	7.83	37.00	14.0–65.0	24.0–51.0	36.6–37.6
CD8	1126	34.04	0.26	8.79	33.00	11.4–65.0	21.5–50.0	33.5–34.6
CD19	1073	14.67	0.17	5.42	14.00	2.2–40.0	7.0–24.0	14.3–15.0
CD16	532	14.58	0.45	10.40	12.20	1.9–69.5	4.5–40.8	13.7–15.5
CD56	501	12.44	0.34	7.55	11.50	0.9–45.0	1.9–25.9	11.8–13.1
CD4/CD8 ratio	1126	1.16	0.01	0.41	1.12	0.2–4.1	0.56–1.95	1.18–1.22

[a]Minimum Maximum of sample values, [b]5th and 95th percentile values of the sample, [c]95% confidence interval.

Table 2. Centre-wise statistical analysis of lymphocyte sub-populations

Lymphocyte sub-population by centre		Number of samples	Mean	SE	SD	Median	Normal range		
							Data range[a]	5th to 95th percentile[b]	Population range[c]
CD3	JNU	151	64.9	0.79	9.8	66.1	30.6–83.1	47.2–79.5	63.3–66.5
	PGI	250	69.9	0.54	8.5	71.0	45.0–96.0	53.0–81.0	68.8–71.0
	AIIMS	153	67.4	0.56	7.0	68.8	42.0–79.0	54.2–76.9	66.3–68.5
	IIH	150	69.8	0.51	6.2	70.1	50.8–84.6	60.1–78.8	68.8–70.8
	CMC	250	69.7	0.54	8.6	71.0	41.0–89.0	54.0–82.0	68.6–70.8
	NARI	172	68.7	0.56	7.4	69.0	48.0–87.0	53.7–79.4	67.6–69.8
CD4	JNU	151	36.37	0.53	6.5	35.8	21.6–51.2	26.0–47.9	35.3–37.4
	PGI	250	39.50	0.54	8.6	39.0	21.0–60.0	28.0–55.4	38.4–40.6
	AIIMS	153	36.29	0.61	7.5	37.0	15.0–52.0	21.0–48.0	35.1–37.5
	IIH	150	37.78	0.44	5.4	37.3	24.0–54.8	28.6–46.2	36.9–38.7
	CMC	250	32.43	0.45	7.0	33.0	14.0–51.0	21.0–44.0	31.5–33.3
	NARI	172	41.15	0.55	7.2	40.2	15.0–65.0	31.0–53.4	40.1–42.3
CD8	JNU	151	26.0	0.46	5.6	25.4	11.4–42.5	17.2–35.9	25.1–26.9
	PGI	250	37.9	0.56	8.9	36.0	21.0–61.0	26.0–59.0	36.8–39.0
	AIIMS	153	29.3	0.60	7.4	28.0	16.0–65.0	20.0–43.3	28.1–30.5
	IIH	150	31.5	0.46	5.6	31.3	16.8–45.0	22.1–40.1	30.6–32.4
	CMC	250	38.3	0.51	8.1	37.0	17.0–65.0	26.0–54.0	37.3–39.3
	NARI	172	35.8	0.57	7.5	36.0	15.0–58.0	25.0–49.0	34.7–36.9
CD19	JNU	146	14.17	0.42	5.1	13.9	4.1–40.0	7.0–22.4	13.3–15.0
	PGI	250	14.83	0.36	5.7	14.0	4.0–38.0	7.0–24.0	14.1–15.6
	AIIMS	105	18.78	0.45	4.6	19.0	8.0–31.0	11.3–26.5	17.9–19.7
	IIH	150	13.02	0.40	5.0	12.7	2.2–25.7	4.8–21.8	12.2–13.8
	CMC	250	12.72	0.30	4.7	12.0	4.0–28.0	6.0–22.0	12.1–13.3
	NARI	172	16.63	0.39	5.1	16.0	5.0–30.0	9.0–26.0	15.8–17.4
CD16	JNU	145	11.24	0.48	5.7	10.0	1.9–29.7	3.6–22.4	10.28–12.2
	PGI	200	12.91	0.45	6.4	12.0	4.0–37.0	4.1–25.9	12.0–13.8
	AIIMS	37	13.43	1.13	6.9	12.0	3.0–31.0	4.8–27.4	11.2–15.7
	IIH	150	20.32	1.26	15.5	14.5	2.5–69.5	6.0–54.1	17.8–22.8
	CMC	–	–	–	–	–	–	–	–
	NARI	–	–	–	–	–	–	–	–
CD56	JNU	151	8.14	0.42	5.1	6.9	0.9–28.3	1.9–19.0	7.3–8.9
	PGI	200	17.91	0.50	7.1	17.0	3.0–45.0	9.0–32.9	16.9–18.9
	AIIMS	–	–	–	–	–	–	–	–
	IIH	150	9.47	0.45	5.5	9.5	0.9–21.9	1.2–17.8	8.6–10.4
	CMC	–	–	–	–	–	–	–	–
	NARI	–	–	–	–	–	–	–	–
CD4/CD8 ratio	JNU	151	1.48	0.04	0.45	1.46	0.64–2.70	0.82–2.33	1.4–1.56
	PGI	250	1.11	0.02	0.38	1.07	0.40–2.09	0.54–1.86	1.07–1.15
	AIIMS	153	1.43	0.04	0.44	1.30	0.70–4.40	0.90–2.03	1.35–1.51
	IIH	150	1.25	0.03	0.33	1.20	0.64–2.67	0.79–1.74	1.19–1.31
	CMC	250	0.90	0.02	0.33	0.86	0.30–2.04	0.41–1.60	0.86–0.94
	NARI	172	1.22	0.03	0.43	1.14	0.47–4.06	0.67–1.94	1.16–1.28

[a]Minimum Maximum of sample values, [b]5th percentile and 95th percentile values of the sample, [c]95% confidence interval.

Results in Table 3 show that the percentage of T-cell population (CD3 population) was comparable in donors from different geographical areas in India, but the mean values for T-cell sub-sets (CD4 and CD8 cells) varied significantly. Thus, donors from the northern and western part of the country (Uttar Pradesh (UP), Punjab, Delhi, Maharashtra) and Chandigarh had relatively high mean values for CD4-T-cells (37 to 40%) and correspondingly lower values for CD8 T-cells. Donors from the southern states, especially Tamil Nadu and Kerala had relatively lower values of CD4 T-cells and higher values for CD8 T-cells. As a result, the CD4/CD8 ratio was significantly higher in the former group compared to the latter group. CD4/CD8 ratios for donors born in Delhi, UP and Punjab were above 1.3, whereas the ratio was 0.91 and 0.99 for Tamil Nadu and Kerala respectively.

Effect of age of the donors on lymphocyte sub-population in their blood samples was examined by categorizing the donors in four age-groups, i.e. < 25 years, 25 to 35 years, 35 to 45 years and > 45 years. Centre-wise data on lymphocyte sub-populations in these four age-groups are shown in Table 4. No apparent age-dependant changes in

Table 3. T-cell population in donors belonging to different states

State of origin of donor	Number of samples	Mean ± SE			
		CD3	CD4	CD8	CD4/CD8 ratio
Uttar Pradesh	64	67.29 ± 0.98	37.44 ± 0.83	28.96 ± 0.88	1.37 ± 0.05
Andhra Pradesh	43	69.91 ± 0.96	35.79 ± 1.07	35.06 ± 1.37	1.11 ± 0.06
Delhi	65	68.36 ± 0.78	37.30 ± 0.93	28.60 ± 0.81	1.38 ± 0.05
West Bengal	31	66.89 ± 1.09	34.95 ± 1.27	30.52 ± 1.40	1.24 ± 0.09
Tamil Nadu	174	69.15 ± 0.66	32.27 ± 0.55	37.78 ± 0.59	0.91 ± 0.03
Maharashtra	223	68.86 ± 0.48	40.09 ± 0.44	33.91 ± 0.46	1.24 ± 0.03
Karnataka	30	70.67 ± 1.50	37.17 ± 1.34	35.59 ± 1.95	1.15 ± 0.07
Kerala	60	69.24 ± 0.99	33.11 ± 0.80	37.01 ± 1.25	0.99 ± 0.05
Punjab	44	68.74 ± 1.12	38.82 ± 1.25	30.41 ± 0.90	1.33 ± 0.06

Table 4. Lymphocyte sub-population in donors of different age groups

Age group (years)	Centre	Number of samples	Mean ± SD				
			CD3	CD4	CD8	CD19	CD4/CD8 ratio
≤ 25	JNU	86	67.06 ± 6.19	36.92 ± 5.89	25.93 ± 4.77	14.20 ± 4.42	1.49 ± 0.42
	PGI	58	72.95 ± 4.11	40.57 ± 9.62	36.64 ± 8.66	13.14 ± 4.78	1.20 ± 0.47
	AIIMS	77	66.58 ± 6.95	35.99 ± 7.14	29.08 ± 6.82	17.76 ± 4.00	1.40 ± 0.37
	IIH	32	71.30 ± 5.71	38.24 ± 4.26	31.72 ± 5.11	11.71 ± 3.03	1.25 ± 0.28
	CMC	96	68.61 ± 8.14	31.12 ± 7.50	38.07 ± 8.24	12.97 ± 5.06	0.87 ± 0.33
	NARI	86	67.06 ± 6.19	36.92 ± 5.89	25.93 ± 4.77	14.20 ± 4.42	1.49 ± 0.42
26–35	JNU	41	69.63 ± 7.56	37.90 ± 7.18	26.54 ± 6.07	14.53 ± 6.29	1.51 ± 0.47
	PGI	149	69.29 ± 9.17	39.62 ± 8.60	38.51 ± 9.33	15.26 ± 5.63	1.09 ± 0.36
	AIIMS	44	68.52 ± 7.01	36.72 ± 7.87	29.19 ± 8.18	19.68 ± 5.06	1.49 ± 0.58
	IIH	67	69.99 ± 6.06	37.41 ± 5.61	31.63 ± 5.63	13.03 ± 5.21	1.23 ± 0.33
	CMC	109	70.44 ± 7.92	32.73 ± 6.46	38.75 ± 7.71	12.24 ± 4.14	0.90 ± 0.32
	NARI	68	68.94 ± 7.84	41.09 ± 6.22	35.59 ± 6.19	16.50 ± 5.01	1.20 ± 0.33
36–45	JNU	18	66.19 ± 13.04	35.33 ± 6.57	37.89 ± 8.78	17.28 ± 8.44	0.97 ± 0.23
	PGI	17	66.34 ± 7.78	34.34 ± 7.62	30.11 ± 7.83	21.76 ± 3.95	1.34 ± 0.31
	AIIMS	31	69.45 ± 5.57	38.49 ± 5.34	30.69 ± 6.14	13.88 ± 5.62	1.31 ± 0.35
	IIH	28	68.89 ± 11.60	34.71 ± 7.30	36.36 ± 8.95	14.36 ± 4.89	1.02 ± 0.39
	CMC	33	69.52 ± 7.11	44.52 ± 7.66	35.15 ± 7.82	16.55 ± 5.21	1.38 ± 0.62
	NARI	18	66.19 ± 13.04	35.33 ± 6.57	37.89 ± 8.78	17.28 ± 8.44	0.97 ± 0.23
> 45	JNU	24	49.01 ± 7.74	31.81 ± 5.89	25.09 ± 7.65	13.23 ± 5.17	1.41 ± 0.51
	PGI	25	68.98 ± 6.45	39.24 ± 6.70	37.20 ± 6.93	14.44 ± 4.61	1.06 ± 0.29
	AIIMS	16	69.77 ± 5.27	38.81 ± 7.86	29.69 ± 8.16	17.44 ± 5.41	1.56 ± 0.39
	IIH	20	67.37 ± 7.74	37.19 ± 6.68	31.69 ± 5.82	13.72 ± 5.40	1.22 ± 0.35
	CMC	17	72.76 ± 8.63	34.18 ± 6.56	40.59 ± 8.83	11.76 ± 5.53	0.89 ± 0.29
	NARI	21	68.62 ± 6.52	43.10 ± 9.66	35.71 ± 8.04	15.19 ± 4.01	1.29 ± 0.52

Table 5. T-cell population in donors above or below 35 years of age

Lymphocyte sub-population	Age group	Number of samples	Mean	SE	SD	*P*
CD4	≤ 35	877	36.9	0.36	7.8	> 0.05
	> 35	249	37.8	0.51	8.0	
CD8	≤ 35	877	34.2	0.30	8.8	> 0.05
	> 35	249	33.6	0.55	8.6	
CD4/CD8 ratio	≤ 35	877	1.18	0.43	0.01	> 0.05
	> 35	249	1.23	0.45	0.03	

Table 6. T-cell populations in male and female donors

Lymphocyte sub-population	Age group	Number of samples	Mean	SE	SD	*P*
CD3	Male	729	68.6	8.5	0.31	> 0.05
	Female	393	68.7	7.7	0.39	
CD4	Male	729	36.3	7.9	0.29	< 0.01
	Female	393	38.6	7.6	0.38	
CD8	Male	729	34.8	9.1	0.34	< 0.01
	Female	393	32.5	8.0	0.40	
CD19	Male	687	14.4	5.3	0.20	< 0.05
	Female	382	15.1	5.6	0.29	
CD4 : CD8	Male	729	1.14	0.02	0.43	< 0.01
	Female	393	1.29	0.02	0.43	

Table 7. Effect of alcohol consumption on lymphocyte sub-populations

Alcohol consumption	Number of samples	Mean ± SD				
		CD3	CD4	CD8	CD19	CD4/CD8 ratio
None	622	68.47 ± 7.76	36.24 ± 7.97	34.13 ± 8.56	14.72 ± 5.30	1.18 ± 0.46
Occasional	143	69.78 ± 7.65	36.27 ± 7.09	34.69 ± 9.08	14.08 ± 5.18	1.15 ± 0.39
Habitual	13	69.12 ± 10.28	37.25 ± 6.71	27.07 ± 6.48	14.10 ± 3.05	1.44 ± 0.38

Table 8. Effect of smoking on lymphocyte sub-populations

Smoking habit	Number of samples	Mean ± SD				
		CD3	CD4	CD8	CD19	CD4/CD8 ratio
None	689	68.69 ± 7.66	36.34 ± 7.78	34.15 ± 8.52	14.73 ± 5.26	1.18 ± 0.46
Occasional	54	69.06 ± 8.74	35.58 ± 7.66	36.49 ± 10.16	13.41 ± 4.58	1.06 ± 0.38
Habitual	36	68.55 ± 8.94	35.96 ± 8.23	29.99 ± 7.77	13.79 ± 5.73	1.30 ± 0.41

Table 9. Effect of smoking habit on lymphocyte sub-populations

Parameter	Self-assessment of donor	Number of donors	CD16 sub-population mean ± SD	*P*
Alcohol consumption	None	198	15.2 ± 12.2	
	Occasional	54	21.4 ± 15.2	$< 0.01^a$
	Habitual	10	17.8 ± 15.0	$> 0.05^a$
Smoking	None	224	16.2 ± 13.0	
	Occasional	16	15.6 ± 10.5	$> 0.05^a$
	Habitual	22	21.1 ± 15.9	$> 0.05^a$
Nutritional Status	Perfect	62	26.5 ± 18.3	
	Good	137	14.3 ± 9.9	$< 0.01^b$
	OK	59	11.8 ± 7.6	$< 0.01^b$
General health	Perfect	55	19.1 ± 15.2	
	Good	153	16.9 ± 13.5	$> 0.05^b$
	OK	52	13.1 ± 8.9	$< 0.05^b$

[a]Significance of difference from control group.
[b]Significance of difference from 'perfect' group.

lymphocyte sub-populations were noted in the data. When the whole data was divided into donors above or below 35 years of age and lymphocyte sub-populations compared in these two-groups, no statistically significant differences occurred between the two age-groups with regard to different T-cell sub-populations (Table 5). Categorization of data based upon sex of the donors indicated a significantly higher CD4 sub-population and a corresponding increase in CD4/CD8 ratio in females (Table 6).

Tables 7 and 8 contain data on lymphocyte sub-population in donors categorized on the basis of alcohol consumption and smoking. Based upon the data, habitual alcoholics have a significantly higher CD4/CD8 ratio ($P = 0.01$), but only 13 donors belonged to the habitual alcoholic group. No difference was noted between the nonalcoholic and occasional alcoholic groups (Table 7). A significant elevation in CD4/CD8 ratio was also noted in habitual smokers (Table 8).

CD16 is a marker associated with NK lymphocytes, even though it is not as specific a marker as are CD3, CD4 and CD8 for T-cells. While extensive multi-parametric statistical analysis was done, some interesting associations between CD16 sub-population and nutritional/habit parameters of donors were apparent. These associations are summarized in Table 9, and indicate that CD16 sub-population increases significantly in subjects who consumed alcohol occasionally and those who assessed their general health and nutritional status as perfect (Table 9).

This study was aimed at defining normal ranges of various lymphocyte sub-populations in blood samples derived from normal healthy Indians. A previous study provided some information on lymphocyte sub-populations in Indians, but the sample size in this study has been low[10]. Moreover, the study has not examined regional differences in lymphocyte counts which may occur in a large country like India. In the current task force-based

study, six centres in different parts of India studied a total of 1027 blood samples from normal healthy Indians using standard flow cytometric methodology.

This study was initiated with the premises that if the equipments and techniques are similar, the reference ranges of lymphocyte populations obtained in different laboratories would also be similar. The inter-laboratory variability encountered in these data suggests that the measurement of the lymphocyte sub-populations using flow cytometry is an ergodic process (processes in which the entire probability distribution is independent of the time and place of generation of data). Other laboratories using such a system can therefore expect to determine similar statistical properties, provided the sample size is sufficiently large. Values for the mean and standard deviation of each lymphocyte sub-population were generated using a Monte Carlo procedure for non-Gaussian distributions and the best-fit distribution. The data can be utilized to assess if a testing laboratory can utilize the normal ranges given in Table 1 as their standard. If means and standard deviations for lymphocyte sub-populations determined in a given testing laboratory, based upon values from a sufficiently large number (say 50) of normal samples, fall within the bounds specified in Table 1, then the reference population of the laboratory is sufficiently similar (with 95% confidence) to the reference ranges reported here.

A comparison of ranges of T- and B-cell sub-populations in healthy Indians (current study) and American standard ranges[2], is given in Table 10. Indian values are essentially similar to the American standards, with some important differences. Indian values for CD4 lymphocytes (T-helper cells) tend to be lower than the corresponding American values. This was further reflected in lower CD4/CD8 ratios for Indians. Detailed analysis for identifying possible sources of variations within our data brought out significantly lower CD4/CD8 ratios for donors belonging to the southern states of Tamil Nadu, Kerala and Andhra Pradesh. Since almost 30% of the samples were from donors belonging to the southern states, the national average of CD4/CD8 ratio also became lower. The CD4/CD8 ratio was significantly higher for the northern and western states of Delhi, Punjab, UP and Maharashtra. These results suggest that significant geographical differences may occur in lymphocyte sub-populations and a relatively lower CD4 value or CD4/CD8 ratio may be normal for subjects from some of the southern states. The reason for these geographical differences is not clear, but may be related to environmental factors, since donors originally from Tamil Nadu and Kerala but living in northern/western states tend to have significantly higher CD4/CD8 ratios (results not shown). While donors from many Indian states were represented in our data, the number of donors from states besides those mentioned in Table 3, was too low to make inter-state comparisons for a large number of large and small states. A much wider study would be needed to thoroughly assess the variations in lymphocyte sub-populations in people residing in different states of India.

Table 10. Comparison of ranges of different lymphocyte sub-populations in healthy Americans and Indians

Lymphocyte sub-population	Mean ± SD	
	American[a]	Indian[b]
CD3	73.0 ± 6.2	68.7 ± 8.2
CD4	43.0 ± 7.5	37.1 ± 7.8
CD8	33.0 ± 7.5	34.0 ± 8.8
CD19	14.0 ± 4.2	14.7 ± 5.4
CD4/CD8 ratio	1.40 ± 0.6	1.16 ± 0.41

[a]Ref. 2; [b]Current study.

Age of the donors did not significantly influence the lymphocyte sub-populations. Significantly higher CD4/CD8 ratios were however found in female donors compared to males. It is possible that sex hormones influence lymphocyte sub-populations and were responsible for the sex-related differences seen in Table 6.

All blood-sample donors were asked to fill a form in which some of the questions were included to obtain a subjective self-assessment of the donor about his/her health status and habits. An interesting correlation was obtained between some of these parameters and percentage of CD16 cells in blood. Donors who considered their health and nutritional status to be very good, had significantly higher CD16 values than those who assessed themselves lower on these parameters. CD16 is a marker associated with NK-cells. Several studies in the literature point out to a positive correlation between blood NK cell and certain 'feel-good' factors[13–15]. Our data along with previous data suggest that the CD16 population of lymphocytes could emerge as a parameter to assess the general health status of donors. Interestingly, consumption of moderate amounts of alcohol was also associated with elevated CD16 values (Table 9). Higher CD16 counts in subjects consuming moderate amounts of alcohol may be correlated with elevated peripheral blood-NK activity demonstrated earlier in such subjects[16]. In experimental studies also, mice provided with alcohol in drinking water had significantly elevated NK levels[17]. Habitual use of alcohol is however not associated with increased CD16 counts.

The current study is the first large multi-centric study sampling subjects from many parts of India for defining the reference ranges of different lymphocyte sub-populations in healthy Indians. While these reference values can be used as standards for Indian populations, further work would be needed to clearly define the nature of differences that prevail in subjects from different areas of the country and the factors responsible for such differences.

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Plant diversity in six forest types of Uttaranchal, Central Himalaya, India

Jeet Ram*, Arvind Kumar and Jitendra Bhatt

Department of Forestry, Kumaun University, Nainital 263 002, India

***Quercus* spp. (oaks) and *Pinus roxburghii* Sarg. (chir-pine) are the major forest-forming tree species in the Central Himalayan region. *P. roxburghii* forest is generally pure with low total species richness of shrubs and herbs, while mixed-broadleaved forest has high total species richness. Shrubs and herbs show high species richness in *P. roxburghii* mixed-broadleaved forest and low species richness in *Quercus semecarpifolia* Sm. forest. *Quercus leucotrichophora* A. Camus forest has high tree diversity, while shrub and herb diversity is highest in *Cupressus – Quercus* mixed forest. Anthropogenic disturbances are changing the species richness and diversity, which influence the soil and environmental conditions. Thus, the conservation and management of these forests will be important for the sustainability of human and land in the region.**

THE most striking feature of the earth is the existence of life, and the most striking feature of life is its diversity[1]. Topography, soil, climate and geographical location of a region influence the vegetation diversity of the forest ecosystem. The Himalayan forest vegetation ranges from tropical dry deciduous forests in the foothills to alpine meadows above timberline[2]. Forest diversity is the main source of livelihood of the people living in Uttaranchal, Central Himalaya. India is among the important mega-biodiversity centres of the world, with a lot of contribution from the Himalayan ecosystem. Biodiversity is used variously for fodder, fuel wood, timber, leaf litter for manuring crop fields, construction, industrial raw material and several non-timber forest produce. Forests of this region are mainly dominated by *Pinus roxburghii* Sarg. (Chir Pine) and *Quercus leucotrichophora* A. Camus. (Banj oak). Chir pine often forms a pure crop in this area, but sometimes it also mixes with certain broadleaved species like *Q. leucotrichophora*, *Quercus glauca* Thumb, *Pyrus pashia* Ham., *Myrica esculanta* Linn. and *Rhododendron arborium* Sm. *Q. leucotrichophora* prefers cooler aspects below 1900 m asl (ref. 3) and is found in either pure or mixed with other broadleaved species.

The increasing population trend over the last few decades and consequent dependence on plant products has led to the vast exploitation of natural flora and fauna of this region. The accelerating effects of human activities on biodiversity might impact ecosystem functioning[4]. This has renewed interest in the effect of diversity on ecosystem[5] and on ecosystem services essential to society[6].

*For correspondence. (e-mail: jeetram2001@yahoo.com)