

Letter to the Editor

Reference values for peripheral blood lymphocyte subsets of healthy children in China

To the Editor:

Immunophenotyping of peripheral blood lymphocyte subsets can provide important information for the diagnosis and treatment of immunological and hematological disorders. Lymphocyte compartments undergo dramatic changes during childhood; age-matched reference values derived from healthy individuals are crucial and have been evaluated in various ethnic

populations.¹⁻⁵ However, extensively detailed immunophenotypic reference values of peripheral blood lymphocytes in whole spectrum of childhood are rare. Our aim was to determine the relative and absolute numbers of lymphocyte subpopulations in healthy Chinese children from birth to age 18 years.

We recruited 1075 Chinese children (604 males and 471 females) who were grouped into 7 categories according to age: group 1, 0 to 28 days; group 2, 1 to 6 months; group 3, 6 to 12 months; group 4, 1 to 4 years; group 5, 4 to 8 years; group 6, 8 to 12 years; and group 7, 12 to 18 years. Whole blood was used and staining for lymphocyte surface markers was performed after red cell lysis, according to a standard flow cytometric multicolor

TABLE I. Distribution of the percentage of total T and B cells and their subsets by age and sex in the peripheral blood of 1075 healthy children (%)

| Subset | Sex | Group 1 0-28 d (n = 21) | Group 2 1-6 mo (n = 104) | Group 3 6-12 mo (n = 97) | Group 4 1-4 y (n = 289) | Group 5 4-8 y (n = 271) | Group 6 8-12 y (n = 158) | Group 7 12-18 y (n = 135) |
|----------------|-----|----------------------------|-----------------------------|-----------------------------|----------------------------|----------------------------|-----------------------------|------------------------------|
| Total T | M | 68.29 (54.26-80.94)* | 63.31 (54.28-71.67) | 64.04 (55.32-73.11) | 65.84 (53.88-72.87) | 68.00 (60.05-74.08) | 66.90 (57.10-73.43)* | 66.94 (56.84-75.02) |
| | F | 75.78 (74.15-82.16) | 66.61 (57.45-75.22) | 67.00 (60.15-72.29) | 64.17 (53.37-71.91) | 67.19 (59.50-75.56) | 70.00 (62.06-76.54) | 68.01 (61.29-73.13) |
| CD4 | M | 47.70 (41.90-55.58)* | 41.96 (33.72-52.43)* | 38.36 (28.17-47.74)* | 35.17 (24.08-42.52) | 33.26 (26.17-40.76)* | 30.21 (24.00-38.72)* | 30.80 (22.25-39.00)* |
| | F | 53.76 (51.37-58.57) | 45.13 (37.71-56.05) | 44.54 (35.23-51.41) | 35.96 (26.19-45.48) | 35.00 (28.49-41.07) | 34.09 (28.47-41.39) | 34.53 (26.36-40.90) |
| CD8 | M | 19.10 (16.94-26.18) | 18.45 (14.08-24.70) | 22.98 (15.88-31.48)* | 25.04 (19.00-32.51) | 26.98 (19.68-34.06) | 27.00 (21.01-33.94) | 28.58 (21.91-36.80) |
| | F | 19.44 (17.27-27.18) | 17.83 (12.61-25.08) | 19.00 (14.11-27.77) | 21.81 (16.29-29.88) | 25.28 (19.70-32.04) | 27.98 (22.50-32.37) | 27.40 (20.99-33.73) |
| Total B | M | 18.69 (7.70-35.29)* | 25.25 (17.34-36.03) | 22.23 (17.20-29.71) | 19.00 (13.23-26.39)* | 14.62 (10.21-20.12) | 13.69 (9.19-19.48) | 13.00 (8.84-17.76) |
| | F | 9.26 (7.49-17.71) | 22.74 (14.71-31.04) | 22.21 (16.57-27.65) | 20.57 (13.93-30.49) | 15.65 (10.46-21.77) | 13.58 (9.23-18.15) | 12.58 (7.73-16.84) |
| NK | M | 8.64 (5.90-15.56) | 9.00 (5.89-14.85) | 8.88 (5.67-15.90) | 12.24 (7.21-20.90) | 14.80 (9.00-22.24) | 17.00 (10.01-26.98)* | 18.06 (10.12-28.34) |
| | F | 8.26 (6.92-14.19) | 8.04 (4.92-13.45) | 8.00 (4.84-15.47) | 11.03 (6.53-22.24) | 13.77 (7.83-20.99) | 13.72 (7.75-23.47) | 17.55 (11.43-27.57) |
| DNT | M | 0.62 (0.33-0.86) | 0.64 (0.33-1.12) | 0.86 (0.41-1.55) | 1.01 (0.37-1.80)* | 1.31 (0.18-2.81) | 1.60 (0.82-2.91)* | 1.48 (0.61-2.31) |
| | F | 0.40 (0.22-0.94) | 0.71 (0.40-1.16) | 1.05 (0.57-1.53) | 1.13 (0.56-2.36) | 1.25 (0.19-2.43) | 1.32 (0.68-2.16) | 1.40 (0.80-2.52) |
| γδT | M | 3.09 (2.54-5.50) | 4.88 (3.32-7.40) | 6.47 (3.95-10.40) | 9.80 (4.94-17.98) | 12.20 (6.92-19.84) | 12.60 (6.92-20.76) | 10.80 (6.55-20.28) |
| | F | 3.33 (2.52-11.47) | 5.58 (3.49-8.29) | 5.77 (3.80-9.20) | 10.20 (5.07-17.60) | 11.30 (7.00-19.60) | 12.30 (7.80-23.35) | 11.45 (6.40-18.50) |
| DPT | M | 0.63 (0.35-3.30) | 0.59 (0.33-0.92) | 0.44 (0.22-1.23) | 0.34 (0.15-1.01) | 0.29 (0.15-0.68)* | 0.41 (0.23-0.94) | 0.37 (0.20-0.80)* |
| | F | 0.79 (0.60-1.56) | 0.62 (0.33-1.17) | 0.53 (0.31-0.82) | 0.37 (0.15-0.96) | 0.39 (0.22-0.71) | 0.51 (0.21-0.92) | 0.55 (0.32-1.08) |
| CD4/CD8 | M | 2.28 (1.98-3.10) | 2.28 (1.47-3.23) | 1.69 (0.93-2.52)* | 1.44 (0.90-2.13)* | 1.25 (0.87-1.94)* | 1.08 (0.81-1.66)* | 1.03 (0.65-1.65)* |
| | F | 2.76 (1.97-3.32) | 2.66 (1.62-3.77) | 2.36 (1.28-3.40) | 1.65 (1.05-2.53) | 1.34 (1.02-2.05) | 1.24 (0.92-1.73) | 1.26 (0.85-1.76) |
| CD4 Naive | M | 87.67 (80.47-93.49) | 82.40 (69.15-88.10) | 78.45 (59.28-88.09) | 70.30 (46.14-84.40) | 62.20 (45.56-75.28) | 57.80 (39.72-69.59) | 53.20 (39.50-66.26) |
| | F | 87.27 (78.15-90.70) | 84.15 (67.52-89.73) | 80.30 (65.58-87.86) | 67.50 (46.42-81.20) | 62.85 (40.75-72.70) | 58.55 (39.85-71.80) | 54.65 (43.30-63.20) |
| CD4 CM | M | 12.11 (6.37-19.02) | 15.10 (10.11-28.20) | 18.40 (10.15-33.38) | 26.40 (13.88-48.12) | 32.90 (22.06-46.46) | 34.55 (24.24-52.73) | 37.80 (25.34-49.90) |
| | F | 12.55 (8.94-21.70) | 14.50 (9.21-32.18) | 16.90 (11.74-32.72) | 28.85 (17.12-47.60) | 32.05 (21.66-52.74) | 35.30 (23.25-51.30) | 36.50 (30.85-45.25) |
| CD4 EM | M | 0.09 (0.05-0.27) | 1.02 (0.28-2.10)* | 1.33 (0.42-3.96) | 2.80 (0.94-6.46) | 4.30 (2.08-8.78) | 6.20 (3.40-11.17)* | 8.10 (4.68-15.70) |
| | F | 0.12 (0.05-0.50) | 0.70 (0.16-1.82) | 1.01 (0.42-2.26) | 2.50 (0.90-5.17) | 4.05 (1.90-9.20) | 5.05 (2.65-9.90) | 6.75 (4.20-16.25) |
| CD4 TEMRA | M | 0.05 (0.02-0.34) | 0.23 (0.00-1.64) | 0.30 (0.00-1.49) | 0.20 (0.00-1.36)* | 0.20 (0.00-1.06) | 0.25 (0.01-1.29) | 0.20 (0.00-1.54) |
| | F | 0.05 (0.01-0.25) | 0.20 (0.00-0.68) | 0.30 (0.00-1.07) | 0.10 (0.00-0.50) | 0.20 (0.00-1.47) | 0.30 (0.07-1.65) | 0.40 (0.10-2.10) |
| CD8 Naive | M | 88.92 (82.14-96.93) | 81.40 (68.90-94.60) | 72.50 (47.36-92.45) | 63.50 (36.80-83.16) | 61.30 (41.58-77.90) | 55.85 (41.41-73.04) | 52.80 (35.34-72.32) |
| | F | 90.20 (84.66-92.62) | 80.45 (63.39-94.29) | 72.70 (53.16-90.14) | 65.40 (38.19-86.18) | 62.00 (38.03-79.08) | 55.05 (36.05-72.25) | 53.90 (37.00-69.35) |
| CD8 CM | M | 10.53 (3.01-16.90) | 13.05 (5.14-25.55) | 11.00 (4.82-24.11) | 15.80 (5.18-31.66)* | 20.60 (12.08-30.54) | 24.95 (13.21-37.89) | 21.00 (10.96-31.00) |
| | F | 8.99 (7.23-11.85) | 13.45 (5.51-27.25) | 11.30 (7.09-31.70) | 20.20 (6.66-34.14) | 22.05 (11.91-36.87) | 26.70 (13.05-39.45) | 24.05 (14.00-36.85) |
| CD8 EM | M | 0.05 (0.03-0.53) | 0.78 (0.10-4.95) | 2.01 (0.20-8.94) | 3.40 (0.70-11.22) | 4.90 (1.58-13.18)* | 5.60 (1.53-15.39) | 7.50 (2.38-15.84) |
| | F | 0.19 (0.06-0.55) | 0.64 (0.02-4.74) | 2.00 (0.18-7.06) | 3.10 (0.60-12.01) | 3.75 (1.11-14.51) | 6.60 (2.00-16.75) | 5.00 (2.40-15.50) |
| CD8 TEMRA | M | 0.12 (0.03-0.73) | 0.65 (0.02-9.61) | 9.30 (0.15-23.32) | 15.50 (0.84-33.02)* | 10.30 (1.70-24.62) | 8.40 (1.01-21.65) | 13.80 (5.08-31.24) |
| | F | 0.24 (0.05-1.16) | 0.34 (0.00-10.48) | 3.63 (0.12-20.70) | 6.75 (0.50-24.45) | 9.80 (1.30-22.85) | 8.60 (1.35-21.50) | 12.75 (3.90-27.25) |
| Naive B | M | 87.20 (77.80-92.90) | 93.15 (87.55-94.85) | 87.80 (75.28-92.77) | 77.60 (65.54-86.62) | 64.50 (48.36-75.84) | 64.85 (51.84-77.61) | 67.26 (53.78-78.64) |
| | F | 88.75 (70.95-93.72) | 92.40 (87.99-94.63) | 87.20 (79.08-93.04) | 76.20 (59.59-85.28) | 62.35 (52.04-75.78) | 64.70 (44.95-75.80) | 68.55 (48.38-77.85) |
| Memory B | M | 2.47 (0.96-3.63) | 2.16 (0.99-4.71) | 3.96 (1.77-7.06) | 7.40 (2.98-14.18)* | 12.90 (7.76-19.90) | 14.60 (8.96-24.09) | 13.50 (7.15-23.10) |
| | F | 2.93 (1.85-20.45) | 2.18 (0.93-4.23) | 4.40 (1.24-7.81) | 7.70 (3.60-18.55) | 14.20 (8.61-20.19) | 13.95 (8.85-22.90) | 15.40 (8.94-23.75) |
| Transitional B | M | 31.60 (9.50-56.50) | 19.90 (15.05-29.95) | 12.60 (6.04-21.62) | 9.80 (5.24-17.22)* | 6.70 (2.58-12.30) | 5.30 (2.50-9.07)* | 3.70 (1.38-9.42) |
| | F | 23.55 (9.83-38.68) | 21.45 (11.77-30.45) | 13.30 (5.84-18.76) | 8.80 (4.73-15.68) | 6.35 (3.41-11.17) | 4.30 (1.75-10.30) | 3.05 (1.35-5.50) |
| Plasmablasts | M | 1.30 (0.50-3.40) | 1.35 (0.60-3.95) | 2.43 (0.71-5.88) | 2.00 (0.50-7.06) | 2.80 (0.90-7.36) | 2.25 (0.70-5.67) | 1.80 (0.49-7.06) |
| | F | 2.74 (0.39-8.06) | 1.70 (0.69-3.42) | 2.80 (0.44-7.40) | 2.20 (0.60-10.31) | 2.45 (0.80-9.75) | 2.20 (0.70-7.95) | 1.75 (0.46-5.80) |

The proportions of CD4 T cells, CD8 T cells, DNT cells, DPT cells, and γδT cells were calculated as percentages of total T cells; subsets of CD4, CD8 T cells and B cells as percentages of CD4, CD8 T or B cells. Values of each population were presented as medians (upper line) and (10th to 90th) percentiles (lower line).

DNT, TCRαβ⁺ CD4 and CD8 double-negative T cell; F, female; M, male; NK, natural killer; TEMRA, terminally differentiated effector memory CD45RA⁺ T cell.

*The differences of values between males and females were statistically significant (Wilcoxon rank sum test was used and a 2-sided *P* value of <.05 was regarded as statistically significant).

TABLE II. The numbers of total T and B cells and their subsets by age and sex in the peripheral blood of 1075 healthy children

| Subset | Sex | Group 1 0-1 mo (n = 21) | Group 2 1-6 mo (n = 104) | Group 3 6-12 mo (n = 97) | Group 4 1-4 y (n = 289) | Group 5 4-8 y (n = 271) | Group 6 8-12 y (n = 158) | Group 7 12-18 y (n = 135) |
|---------------------------------------|-----|----------------------------|-----------------------------|-----------------------------|----------------------------|----------------------------|-----------------------------|------------------------------|
| Lymphocytes ($\times 10^9/L$) | M | 4.47 (3.47-6.63) | 5.31 (3.68-7.34) | 5.89 (3.73-8.76) | 4.44 (2.79-6.35) | 2.94 (2.28-3.82)* | 2.53 (2.02-3.61) | 2.44 (1.78-3.44) |
| | F | 4.44 (3.27-6.11) | 5.12 (4.02-6.45) | 5.71 (3.78-8.11) | 4.39 (2.98-5.95) | 3.04 (2.37-4.29) | 2.65 (2.02-3.50) | 2.49 (1.76-3.00) |
| Total T (cells/ μL) | M | 3073 (1856-4021) | 3488 (2179-4424) | 3595 (2187-6352) | 2843 (1794-4247) | 1989 (1424-2664) | 1686 (1325-2276)* | 1661 (1184-2144) |
| | F | 3636 (2421-4577) | 3369 (2766-4068) | 3625 (2488-5422) | 2778 (1775-3953) | 2092 (1480-2847) | 1864 (1297-2480) | 1617 (1169-2071) |
| CD4 (cells/ μL) | M | 2156 (1330-3105) | 2279 (1461-3018) | 2188 (1125-3768) | 1467 (902-2253) | 960 (686-1358)* | 794 (531-1110)* | 776 (522-1084) |
| | F | 2548 (1744-3226) | 2287 (1890-2988) | 2539 (1433-3874) | 1518 (948-2477) | 1066 (767-1592) | 897 (621-1258) | 843 (554-1109) |
| CD8 (cells/ μL) | M | 880 (657-1152) | 979 (556-1687) | 1396 (686-2278)* | 1131 (580-1735)* | 754 (518-1125) | 690 (480-1112) | 713 (489-1009) |
| | F | 1026 (609-1348) | 956 (658-1276) | 1059 (710-1843) | 979 (531-1521) | 773 (553-1127) | 714 (509-1050) | 672 (423-900) |
| Total B (cells/ μL) | M | 774 (344-2090) | 1234 (734-2265) | 1302 (916-1832) | 822 (461-1456) | 423 (280-623)* | 350 (216-536) | 316 (203-476) |
| | F | 466 (292.26-858.94) | 1058 (667.15-2044.69) | 1226 (807.44-1803.72) | 867 (537.11-1464.39) | 473 (303.52-777.25) | 333 (247.05-578.16) | 309 (176.56-415.64) |
| NK (cells/ μL) | M | 350 (267-730) | 471 (290-780) | 588 (306-896)* | 508 (270-1053) | 406 (258-727) | 423 (246-792)* | 425 (210-804) |
| | F | 377 (266-602) | 411 (221-722) | 416 (243-924) | 473 (241-978) | 432 (227-668) | 366 (203-584) | 404 (232-789) |
| DNT (cells/ μL) | M | 20 (11-26) | 18 (11-45) | 29 (16-58) | 27 (9-57)* | 23 (4-55) | 26 (13-48) | 23 (12-37) |
| | F | 17 (6-35) | 23 (13-38) | 37 (19-72) | 31 (16-58) | 26 (4-49) | 23 (12-41) | 22 (13-44) |
| $\gamma\delta T$ (cells/ μL) | M | 97 (51-240) | 141 (92-279) | 238 (128-436) | 267 (114-539) | 233 (124-410) | 210 (124-388) | 198 (81-343) |
| | F | 139 (71-356) | 187 (94-301) | 205 (143-409) | 283 (128-520) | 243 (134-428) | 234 (121-462) | 176 (85-358) |
| DPT (cells/ μL) | M | 34 (16-121) | 28 (16-56) | 25 (9-67) | 16 (6-50) | 9 (4-21)* | 10 (6-26) | 9 (4-21)* |
| | F | 37 (30-70) | 31 (19-73) | 29 (13-50) | 15 (6-50) | 12 (6-25) | 13 (5-29) | 13 (7-30) |
| CD4 Naive (cells/ μL) | M | 1943 (1182-2585) | 1839 (1170-2595) | 1802 (764-2972) | 918 (472-1760) | 595 (321-972) | 407 (294-683)* | 410 (230-627) |
| | F | 1982 (1592-2853) | 1908 (1433-2546) | 1967 (1042-3160) | 929 (530-1837) | 633 (339-1037) | 489 (299-857) | 442 (276-654) |
| CD4 CM (cells/ μL) | M | 227 (145-387) | 318 (213-647) | 362 (206-796) | 377 (212-735) | 305 (211-478)* | 268 (165-475)* | 257 (182-403) |
| | F | 339 (162-544) | 341 (239-676) | 384 (236-803) | 448 (220-800) | 341 (232-601) | 317 (219-463) | 296 (203-422) |
| CD4 EM (cells/ μL) | M | 3 (1-6) | 21 (5-48)* | 29 (11-60) | 41 (15-87) | 40 (23-84) | 45 (24-87) | 60 (29-117) |
| | F | 3 (2-13) | 16 (3-42) | 23 (12-56) | 33 (14-93) | 41 (20-97) | 42 (24-94) | 53 (31-128) |
| CD4 TEMRA (cells/ μL) | M | 2 (1-4) | 5 (0-40) | 5 (0-33) | 4 (0-22)* | 1 (0-13)* | 2 (0-9) | 2 (0-12) |
| | F | 2 (0-4) | 4 (0-16) | 7 (0-23) | 2 (0-12) | 2 (0-17) | 2 (1-15) | 3 (1-18) |
| CD8 Naive (cells/ μL) | M | 775 (535-1054) | 800 (503-1276) | 909 (535-1677)* | 653 (356-1095)* | 462 (297-730) | 380 (245-657) | 375 (231-568) |
| | F | 892 (519-1205) | 741 (484-1009) | 726 (461-1235) | 589 (295-971) | 447 (293-768) | 387 (232-665) | 328 (210-560) |
| CD8 CM (cells/ μL) | M | 101 (29-140) | 124 (41-305) | 165 (51-316) | 156 (56-406) | 158 (85-268) | 179 (92-287) | 152 (74-228) |
| | F | 93 (45-127) | 124 (46-256) | 131 (59-381) | 177 (54-379) | 173 (80-350) | 178 (100-301) | 154 (87-275) |
| CD8 EM (cells/ μL) | M | 1 (0-5) | 6 (1-70) | 28 (2-120) | 39 (6-145)* | 38 (10-129) | 40 (9-130) | 55 (16-109)* |
| | F | 2 (1-4) | 6 (0-52) | 22 (2-116) | 28 (4-163) | 28 (8-104) | 45 (14-157) | 35 (13-110) |
| CD8 TEMRA (cells/ μL) | M | 1 (0-6) | 5 (0-133) | 146 (2-430)* | 154 (9-440)* | 80 (11-218) | 56 (12-164) | 100 (29-269) |
| | F | 2 (1-13) | 3 (0-109) | 54 (1-220) | 75 (3-294) | 69 (9-210) | 64 (11-175) | 68 (20-218) |
| Naive B (cells/ μL) | M | 675 (268-1674) | 1157 (691-2132) | 1146 (726-1626) | 629 (323-108) | 264 (147-431)* | 233 (123-362) | 206 (116-347) |
| | F | 432 (196-801) | 947 (597-1941) | 1032 (729-1474) | 677 (371-1197) | 295 (171-469) | 203 (140-381) | 185 (98-296) |
| Memory B (cells/ μL) | M | 15 (8-67) | 29 (12-54) | 51 (22-103) | 52 (26-124)* | 51 (31-94)* | 52 (28-89) | 40 (20-86) |
| | F | 20 (14-48) | 25 (10-44) | 52 (16-124) | 72 (33-146) | 64 (38-115) | 51 (30-90) | 42 (27-70) |
| Transitional B (cells/ μL) | M | 282 (33-526) | 250 (136-464) | 164 (65-288) | 78 (35-172) | 27 (10-66) | 18 (7-37) | 11 (4-37) |
| | F | 95 (36-384) | 223 (122-445) | 172 (74-266) | 70 (33-181) | 28 (14-60) | 15 (5-37) | 9 (3-19) |
| Plasmablasts (cells/ μL) | M | 12 (2-27) | 19 (6-42) | 33 (9-72) | 17 (4-63) | 11 (4-28) | 7 (3-21) | 6 (1-23) |
| | F | 18 (1-29) | 18 (8-36) | 30 (6-109) | 23 (4-88) | 11 (4-40) | 8 (2-36) | 5 (1-15) |

The total numbers of white blood cells, lymphocytes, and T cells, B cells, NK cells, and their subsets for each age group are shown as the median (upper line) and (10th to 90th) percentiles (lower line).

DNT, TCR $\alpha\beta^+$ CD4 and CD8 double-negative T cell; F, female; M, male; NK, natural killer; TEMRA, terminally differentiated effector memory CD45RA⁺ T cell.

*The differences in values between males and females were statistically significant (Wilcoxon rank sum test was used and a 2-sided *P* value of <.05 was regarded as statistically significant).

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