Reference values of lymphocyte subpopulations in European adolescents. Preliminary results from the HELENA study

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The cell surface markers of peripheral blood lymphocytes have provided extraordinary information about the maturation and activation of the human immune system; however, little is known about the changes in lymphocyte subsets during the stage of adolescence, which is characterised by an intense growth and sexual maturation with underlying involvement of the neuroimmunoendocrine system.

The aim of the current study was to determine the distribution of lymphocyte subsets in healthy adolescents in the HELENA Cross-Sectional Study (CSS). From the total population included in the HELENA study (CSS) a sub-sample of 1000 adolescents (between 12.5 and 17.5) who underwent blood extraction were studied. Whole blood was collected in EDTA tubes and aliquoted in one eppendorf, diluted 1:1 with Cytochex™ Reagent (Streck Laboratories, Omaha, NE, USA). All samples were analysed within 7 days after blood withdrawal. Briefly, blood samples were incubated for 30 min at room temperature with monoclonal antibodies purchased from BD Biosciences (San José, CA, USA) by four-staining procedure (multitests CD3+/CD16+56+/CD45+/CD19+ and CD3+/CD8+/CD45+/CD4+ and the combinations CD3+/CD4+/CD45RA+/CD45RO+ and CD3+/CD8+/CD45RA+/CD45RO+). After the lysis of red cells, lymphocytes were gated by forward and side scatter and pan-leucocyte marker expression (CD45+) and analysed by flow cytometry (FACSCAN PLUS DUAL LASER, Becton Dickinson Sunnyvale, CA). Subjects were classified in age category (12.5–13.99, 14–14.99, 15–15.99, 16–17.50), and BMI (underweight [<18.5]; normal weight, overweight and obese) groups and one-way ANOVA was performed considering each of these fixed factors independently.

Among the basic lymphocyte subsets no differences were found by age in T lymphocyte subsets but significant differences were found for CD19+ (% and cell numbers) and CD(16+56)+ (percentage). CD19+ subset (B lymphocytes) decreased with age, while the CD(16+56)+% increased with age. The CD3/CD19 ratio also increased with age. Regarding naïve and memory T lymphocytes, the CD4+CD45RA+ (naive) cells decreased and the CD4+CD45RO+ (memory) cells increased with age (percentage and counts; P between 0.004 and <0.001). The CD8+CD45RA+ and CD8+CD45RO+ subsets showed similar trends, while it was only significant in percentage ($P = 0.01$). Differences in the percentage of some lymphocyte subsets by weight status were found. The CD4+CD45RA+ cells were lower in the obese group compared with the normal weight group (53.20 ± 11.32 *v.* 57.33 ± 10.57, respectively), while the CD4+CD45RO+ cells were higher in the overweight group (44.77 ± 10.23) compared with the normal weight group (42.26 ± 10.64). The increase in CD4+CD45RO+ cells was also observed as a non-significant trend in the obese group.

In conclusion, the data obtained in this study would provide reference lymphocyte subset values that might be useful for comparison with data obtained in other healthy as well as diseased European adolescent populations.