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Parental education associated with immune function in adolescence

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Background: The immune system is affected and shaped by several internal and external factors. Among the external variables, the socioeconomic status is known to influence the immune system since the early years of life and throughout life. **Methods:** In this study, we assessed the relationship between parental education with the white blood cells and its subtypes in 1213 adolescents from the *EPITeen* cohort, assessed at the age of 13. Beta coefficients (β) and 95% confidence intervals (CI) were fitted using linear regression models to quantify the association and were adjusted for sex, body mass index and chronic disease. **Results:** After adjustment, parental education presented a negative association with white blood cells, which was significant among those with higher *high-sensitivity* C-reactive protein (*hs*-CRP) median levels [−0.05 mg/l (95% CI −0.08, −0.01)]. On the contrary, a positive association with lymphocytes was observed, which was, significant among those with lower *hs*-CRP [0.17 mg/l (95% CI 0.02, 0.32)]. A neutrophil-to-lymphocyte ratio significant decrease was also observed with the increment of parental education ($P<0.001$). **Conclusion:** We found that parental education was positively associated with a higher proportion of lymphocytes and a lower proportion of neutrophils, suggesting that parental education is associated with offsprings innate immune system regulation. These results may contribute to clarify the relationships between childhood socioeconomic status and increased risk of adverse cardiovascular outcomes and other immune-related diseases.

Introduction

The immune response, either innate or adaptive, is a human physiological function that aims to maintain bodýs health against internal and external aggressions.[1,2] Among the external variables, the social and environment exposures have been shown to influence and modulate the immune system since the early years of life, throughout childhood and adolescence and into adulthood.[3–7]

Although the biological mechanisms associated with these effects are not well-understood different approaches have shown that social and environmental stressors, can induce pro-inflammatory responses, including production of interleukin-6 and C-reactive protein (CRP).[3,8,9] Moreover, evidence from non-human primate models suggests that changes on social environment can affect the regulation of genes controlling physiologic functions, namely the immune system.[9]

The increased inflammatory response is thought to be due to the chronic or repeated stimulation of the sympathetic nervous system, as well as to progressive down-regulation of key anti-inflammatory pathways, such as the hypothalamic–pituitary–adrenal axis and the parasympathetic nervous system.[10,11] In addition to elevated inflammatory levels, chronic stress exposure has shown to be associated with impaired cell-mediated acquired immunity.[2]

Since socioeconomic status (SES) is one of the major determinants of social and environment exposures, can also be expected to influence the immune system response. Individuals with a lower educational status have shown to be exposed to a greater chronic stress and to have higher levels of immune mediators such as CRP, fibrinogen or monocyte chemoattractant protein-1.[3,8,12,13] Therefore, the increase on these immune mediators may lead to a higher risk of adverse health outcomes, namely cardiovascular disease, cancer and other immune-related diseases.[3,12,14]

Several studies have described the so called allostatic load as the biological 'wear-and-tear' resulting from chronic exposure to social and environmental stressors.[6,7] This concept of allostasis, by Sterling and Eyer, means 'stability through change', in which an organism must vary parameters of its internal milieu and match them appropriately to environmental demands. In fact, the biological risk can be more effectively conceptualized from a cumulative view and that such conception can better contribute to our understanding of health outcomes.[6,7] Furthermore, the long-term effect of socioeconomic factors on the immune response is carried out through cellular and humoral mediators in a highly coordinated fashion, but it is not well understood.[15]

In this study, we aimed to investigate whether social factors during early life could affect and tune the immune function, using parental education as a surrogate of SES for the early years of live,[16,17] by evaluating its relationship with white blood cells (WBCs) and its subtypes in adolescents.

Methods

Participants were adolescents with 13 years old from the *Epidemiological Health Investigation of Teenagers* in Porto (EPITeen). As already reported elsewhere,[18] the EPITeen cohort evaluated

adolescents born in 1990, who were enrolled at public and private schools in Porto, Portugal, during the 2003/2004 school year. The Ethics Committee of Hospital S. João approved the study and written consent was obtained from both legal guardians and adolescents.

Subjects

We identified 2786 eligible participants, of whom 2159 agreed to participate and provided information at least for part of the planned assessment, resulting in an overall participation rate of 77.5% [similar in public (77.7%) and private (77.0%) schools ($P = 0.709$)]. Of the 2159 participants, 772 participants did not perform the blood measurements, 159 had missing data on haematological parameters and 15 presented high-sensitivity CRP (*hs*-CRP) concentrations $\geq$10 mg/l, which might be indicative of acute infection.[19] Thus, the analysis was based on the information of 1213 participants.

Participant's characteristics were compared between included and non-included participants. Non-included participants were mostly from public schools, with less educated parents, and a higher proportion involved in more intense physical activity in leisure-time activities. No significant differences were found regarding sex and body mass index (BMI).

Data collection

Data were collected using two self-administered questionnaires: one filled in at home, comprising information on demographic and social characteristics, family and individual history of disease; another questionnaire was filled in at school, which included information on physical activity, smoking and alcohol intake. A physical examination was performed at school, by a team of experienced nurses, nutritionists and physicians, comprising anthropometric assessment and a venous blood sample drawn after a 12-h overnight fast.

Parental education was assessed by asking the parents the question: 'What is the last year of formal schooling you have completed?' and in the analysis we used the information from the parent with the higher education level.

Chronic disease was assessed by asking parents the question 'Does your child currently have any illness requiring regular medical care?' If the answer was positive, information was gathered on type of disease.

Anthropometrics

Weight and height were obtained with the subject in light indoor clothes and barefoot. Weight was measured in kilograms, to the nearest 10th, using a digital scale (Tanita TBF-300, Tanita Corporation of America, Inc., Illinois, USA) and height was measured in centimetres, to the nearest 10th, using a portable stadiometer. Adolescents were classified according to the age- and sex-specific BMI based on the Centers for Disease Control and Prevention's 2000 BMI-for-age growth charts for the USA.[20] Based on this data we classified those above the 95th percentile as obese and those between the 85th and 95th percentiles as overweight.

Blood measurements

A venous blood sample was drawn after an overnight fast. All the samples were analyzed at the central laboratory of the Hospital S. João. Serum *hs*-CRP was determined through particle-enhanced immunonephelometry using an auto-analyser Behring Nephelometer II, BNII® (Dade Behring Marburg GMBH, Germany). Total WBC and its subtypes (neutrophils, monocytes, lymphocytes, eosinophils and basophils) were obtained using an automated blood counter Sysmex®XE-5000 (Hyogo, Japan).

Statistical analysis

The distribution of quantitative variables was checked and, in general, results were presented as mean (standard deviation) and compared through the *t*-test, one-way ANOVA but as eosinophils and basophils follow a non-parametric distribution, they were presented by median (25th–75th percentiles) and compared through Mann–Whitney U test or Kruskal–Wallis. To evaluate the association between parental education levels and the WBC and its subtypes, parental education was used as a continuous variable, and linear regression models were fitted to estimate beta coefficients (β) and 95% confidence intervals (CI). The analyses were stratified in two categories of *hs*-CRP levels considering those below and above the median, and adjusted for sex, BMI and chronic disease.

Statistical analyses were performed using the Statistical Package for the Social Sciences (IBM® SPSS Statistics), version 23.0 and statistical significance was considered with an alpha critical value of 0.05.

Results

Haematological values according to sample characteristics are described in table 1. A significant decrease in total leukocytes and in the proportion of neutrophils was found with the increase of parental educational levels ($P < 0.001$). On the other hand, a significant increase was observed on the lymphocytes proportion with increasing parental education ($P = 0.001$). In general, the percentage of the different WBC subtypes was lower in females, apart from neutrophils that was lower among males. Subjects with higher BMI had significant higher levels of total WBC ($P = 0.006$). Adolescents with higher *hs*-CRP levels showed higher levels of total WBC and higher percentage of its subtypes, except for lymphocytes. Subjects with chronic disease had significant higher percentage of eosinophils and lower percentage of monocytes.

In this study, we reported a significant decrease in neutrophil-to-lymphocyte ratio (NLR) with the increment of parental education ($P < 0.001$) (table 1).

The association between parental education with the WBC and its subtypes, stratified for median *hs*-CRP levels, is presented in table 2. After adjustment, for each additional year of parental education, WBC decreased 0.02 mg/l (95% CI −0.04, 0.00) in adolescents with *hs*-CRP lower than 0.3 and 0.05 mg/l (95% CI −0.08, −0.01) in subjects with *hs*-CRP equal or higher than 0.3 mg/l. In addition, neutrophils decreased with parental education [0.18 mg/l (95% CI −0.34, −0.01) in subjects with lower *hs*-CRP and 0.14 mg/l (95% CI −0.35, 0.08) in subjects with higher *hs*-CRP]. On the other hand, lymphocytes increased with parental education [0.17 mg/l (95% CI 0.02, 0.32) in the subjects with lower *hs*-CRP and 0.12 mg/l (95% CI −0.07, 0.31) in the subjects with higher *hs*-CRP]. For the remaining cell subtypes no significant association was found with the parental education.

Discussion

In this study, we found that parental education was positively associated with a higher proportion of lymphocytes and a lower proportion of neutrophils. Since, lymphocytes, particularly CD4+T cells, represent the regulatory arm of the immune system, and are involved in the suppression of inflammation (through the secretion of anti-inflammatory cytokines and other mechanisms),[14,21] these results suggest that the immune system of adolescents with higher educated parents, may have been moved towards an immunosuppressive phenotype. Moreover, the total WBC counts (a pro-inflammatory marker) were also diminished for these subjects, independently of *hs*-CRP concentrations.

Although we excluded those with *hs*-CRP levels compatible with acute inflammatory episodes ($\geq$10 mg/l), since the remain higher values of *hs*-CRP may indicate a more pronounced activation of the inflammatory response than in participants with values of *hs*-CRP below the median, were stratified by *hs*-CRP median levels. In general, the associations persisted after stratification. These results may

Table 1 WBCs and its subtypes according to the participants' characteristics

	WBC ($\times 10^9$/l)		%Eosinophils	%Basophils	%Monocytes	%Neutrophils	%Lymphocytes	NLR	*hs*-CRP
	n	Mean (SD)	Median (P25–75)	Median (P25–75)	Mean (SD)	Mean (SD)	Mean (SD)	Median (P25–75)	Median (P25–75)
Overall	1213	6.28 (1.58)	2.50 (1.50–4.30)	0.30 (0.20–0.50)	7.22 (2.01)	53.0 (10.5)	36.0 (9.5)	1.45 (1.10–1.95)	0.30 (0.10–0.80)
PE (years)	1180								
≤4	301	6.61 (1.78)	2.40 (1.45–4.20)	0.30 (0.20–0.50)	7.27 (1.99)	54.6 (10.9)	34.4 (10.0)	1.60 (1.14–2.22)	0.30 (0.20–0.80)
5–9	251	6.37 (1.67)	2.20 (1.40–3.90)	0.30 (0.20–0.40)	7.09 (2.08)	54.2 (10.6)	35.1 (9.9)	1.54 (1.11–2.09)	0.40 (0.20–0.90)
10–12	316	6.13 (1.47)	2.40 (1.40–4.18)	0.30 (0.20–0.50)	7.12 (2.01)	52.1 (10.9)	36.9 (9.6)	1.38 (1.06–1.86)	0.20 (0.10–0.70)
≥13	312	6.06 (1.35)	2.60 (1.60–5.00)	0.40 (0.20–0.50)	7.41 (2.00)	51.6 (9.4)	36.9 (8.6)	1.35 (1.06–1.83)	0.20 (0.10–0.70)
P-value		**<0.001**	0.14	0.24	0.19	**<0.001**	**0.001**	**<0.001**	**0.001**
Sex	1213								
Female	644	6.51 (1.62)	1.90 (1.23–3.40)	0.30 (0.20–0.50)	6.96 (1.98)	55.6 (10.4)	34.2 (9.6)	1.64 (1.06–2.00)	0.30 (0.10–0.60)
Male	569	6.02 (1.49)	3.10 (1.90–5.50)	0.30 (0.20–0.50)	7.52 (2.00)	50.0 (9.8)	37.9 (9.1)	1.25 (0.98–1.71)	0.30 (0.20–0.90)
P-value		**<0.001**	**<0.001**	0.65	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**0.003**
BMI	1211								
<85th	897	6.23 (1.64)	2.50 (1.50–4.55)	0.30 (0.20–0.50)	7.23 (1.99)	52.9 (10.5)	35.9 (9.6)	1.44 (1.06–2.00)	0.20 (0.10–0.50)
85–95th	199	6.21 (1.34)	2.60 (1.40–4.00)	0.30 (0.20–0.50)	7.44 (2.14)	52.2 (11.4)	36.4 (10.3)	1.44 (1.07–2.04)	0.40 (0.20–0.90)
≥95th	115	6.72 (1.36)	2.20 (1.40–3.70)	0.30 (0.20–0.40)	6.82 (1.84)	54.5 (8.8)	35.5 (7.4)	1.46 (1.21–1.95)	1.00 (0.40–2.10)
P-value		**0.006**	0.20	0.34	**0.029**	0.18	0.69	**<0.001**	**<0.001**
hs-CRP	1213								
<0.3	679	6.01 (1.35)	2.40 (1.50–4.40)	0.30 (0.20–0.50)	7.20 (1.96)	51.7 (10.2)	37.3 (9.1)	1.38 (1.02–1.87)	
≥0.3	534	6.63 (1.77)	2.60 (1.40–4.23)	0.30 (0.20–0.50)	7.25 (2.07)	54.6 (10.7)	34.3 (9.8)	1.56 (1.17–2.15)	
P-value		**<0.001**	0.92	0.26	0.69	**<0.001**	**<0.001**	**<0.001**	
Chronic disease	1139								
No	898	6.26 (1.53)	2.30 (1.48–4.00)	53.1 (10.2)	36.1 (9.5)	7.29 (2.03)	0.30 (0.20–0.50)	1.45 (1.08–1.98)	0.30 (0.10–0.70)
Yes	241	6.37 (1.73)	3.10 (1.45–6.20)	52.6 (11.6)	35.5 (9.6)	6.95 (1.91)	0.30 (0.20–0.50)	1.43 (1.05–2.07)	0.30 (0.20–0.80)
P-value		0.33	**0.021**	0.51	0.37	**0.021**	0.92	0.88	0.15

PE: parental education; BMI: body mass index; WBC: white blood cell; NLR: neutrophils-to-lymphocytes ratio; *hs*-CRP: *high-sensitivity* C-reactive protein; P25–75: 25th–75th percentiles. Statistically significant *P*-values are in bold.

Table 2 Association between parental education with the WBCs and its subtypes stratified by *hs*-CRP median and adjusted for sex, BMI and chronic disease

	Crude model		***Multivariable model***[a]	
	***hs*-CRP**		***hs*-CRP**	
	<0.3 mg.l^{-1} B (95% CI)	**≥0.3 mg.l^{-1} B (95% CI)**	**<0.3 mg.l^{-1} B (95% CI)**	**≥0.3 mg.l^{-1} B (95% CI)**
WBC	**−0.03 (−0.05, 0.00)**	**−0.06 (−0.09, −0.02)**	**−0.02 (−0.04, 0.00)**	**−0.05 (−0.08, −0.01)**
Neutrophils	**−0.23 (−0.40, −0.06)**	**−0.23 (−0.44, −0.02)**	**−0.18 (−0.34, −0.01)**	−0.14 (−0.35, 0.08)
Eosinophils	0.01 (−0.01, 0.02)	0.01 (0.00, 0.03)	0.00 (−0.01, 0.01)	0.01 (−0.01, 0.02)
Basophils	0.01 (0.00, 0.02)	0.01 (−0.01, 0.02)	0.01 (−0.01, 0.02)	0.01 (−0.01, 0.02)
Lymphocytes	**0.20 (0.05, 0.35)**	0.17 (−0.02, 0.36)	**0.17 (0.02, 0.32)**	0.12 (−0.07, 0.31)
Monocytes	0.01 (−0.02, 0.04)	0.02 (−0.02, 0.06)	0.00 (−0.03, 0.03)	0.01 (−0.03, 0.05)

a: Multivariable model adjusted for sex, BMI and chronic disease. Statistically significant associations are in bold.
WBC: white blood cell; *hs*-CRP: *high-sensitivity* C-reactive protein. The parental education is the exposure variable expressed as a continuous variable.

indicate that parental education triggers immune system regulation independently from *hs*-CRP levels, an acute phase protein used as marker of low-grade inflammation. The final model was adjusted for sex, BMI and chronic disease. The choice of these confounders was based on statistical criteria and on the theoretical relevance of these issues for the association. Both, chronic disease and BMI are related with SES, being those from lower SES the ones with worse profiles.[12] Actually, chronic disease may lead to the immune system up-regulation and several lines of evidence suggests a close link between inflammation and many chronic conditions, such as cardiovascular disease, cancer, rheumatoid arthritis, inflammatory bowel disease and asthma, the latter the most common chronic condition in adolescence.[22] In fact, in chronic diseases occurs an increase of the inflammatory response in which immune cells loss their self-limiting nature and become dysregulated leading to significant tissue or organ damage and limited tissue repairing.[22] On the other hand, the increment in BMI (related with adiposity) also drives to an increase in the systemic inflammation due to the release of pro-inflammatory mediators.[23] Here, the results remained similar after the adjustments, suggesting that mechanisms additional to those regarding to impaired adipocytés metabolism may be involved on the long-term modulation of the immune system. With regard to inflammation, the traditional features of this state classically described as the principal response of the body invoked to deal with injuries (short-term response) is a crucial component of tissue repair and involves integration of many signals in distinct cells and organs. However, the long-term consequences of prolonged inflammation represent a distinct form of response implicating a complex integration of regulatory systems with the involvement of several distinct mediators.[24]

In this sense, an increased neutrophil count and a lower lymphocyte count, defined as NLR ratio, has been recently reported as marker for general immune responses to various stress stimuli.[25–28] Neutrophils secrete several mediators including elastase, myeloperoxidase, various hydrolytic enzymes and oxygen-free radicals that are involved in innate immune response and increase of systemic

inflammation.[25–28] In our study, we reported an NLR increase with the decrement of the parental education, which may indicate that subjects with lower parental education could be under higher chronic stress and, therefore, increased low-grade inflammation. Nevertheless, it is still unclear whether the NLR is merely a marker of poor prognosis or whether it plays a substantial part in the pathogenesis of the adverse outcomes.[25–28]

In accordance with the literature, herein girls presented more total WBC and neutrophils while boys had more lymphocytes, monocytes and eosinophils.[29] Furthermore, some studies reported sex-differences for the increase of immune markers due to lower the parental education, indicating a stronger effect in girls.[30] In this work, we have reported no significant sex-differences, hinting that the mechanisms by which this SES component influences WBC and its subtypes levels are similar for both sexes.

Our results might suggest that this proxy of SES could influence the regulation of the immune system since early life. In fact, SES is a determinant of early-childhood environment, such as air pollutants, viral infections, maternal smoking, psychological stress and allergen exposure as well as more proximal behavioural mechanisms, such as health related practices including smoking, physical activity, dietary choices, and attendance to religious practices.[31–36] Therefore, it might modulate immune development as well as a wide range of metabolic and endocrine systems during the early stages of life, thereby influencing health in adulthood.[37–40] From an allostatic perspective, the chronic exposure to these stimuli either environmental or more proximal, will influence and modulate several endogenous systems, such as the immune system throughout life. The global pathophysiological mechanism by which SES, namely parental education, affects systemic inflammation is still unclear. However, it may be related with unfavourable material conditions associated with chronic stress. Therefore, these adverse circumstances could play a significant role in the aetiology of inflammation through endocrine pathways involving the hypothalamic–pituitary–adrenal axis or the activation of the sympathetic nervous system.[12,31,32,41,42]

Nevertheless, these findings should be interpreted in light of some limitations. The participants who did not accept perform blood measurements, in general are similar to those included in our study, but were mostly from public schools and with less educated parents representing a potential selection bias. However, we can assume that the estimate of association between parental education and WBC and its subtypes is valid since the greatest impact of this loss of participants are the loss of variability in the sample in relation to the exposition (parental education) and the loss of statistical power. Both consequences contribute to reduce the ability to find statistically significant associations but may have limited modifying the direction of the association. Moreover, the cross-sectional design of this study hinders our ability to infer causality. Though it is widely accepted that the chronicity of the low-grade immune system activation across the early stages of life is a key feature in the onset of cardiovascular complications in adulthood, it should be noteworthy in the future to test a prospective approach in order to assess whether this association persists during adolescence and early adulthood. Likewise, we suggest that chronic stress and health-promoting resources should be assessed on inflammatory mediators to help unveiling how parental education may affect individual health. Finally, one should also bear in mind that parental education may not measure SES accurately and other indicators of family social position, such as parental occupation and income, may add other information. However, in Portugal, occupation is strongly related with education and income being more related with the actual situation. Thus parental education may better reflect the global exposure of the offspring throughout childhood.

In conclusion, this study might contribute to a better understanding of the relationships between parental education and haematologic components involved in the immune response, since, according to our knowledge, this has not yet been established for this type of apparently healthy population. Notwithstanding, our findings, based on a population-based sample of adolescents, provide evidence that parental education is associated with offsprings innate immune system regulation and, therefore, may be a possible explanation for the relationships between childhood SES and the increased risk of adverse cardiovascular outcomes and other immune-related diseases. Further research to clarify this potential pathway is warranted.

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Conflicts of interest: None declared.

Key points

- Parental education is associated with offsprings immune system regulation.
- Parental education could be involved in the relationships between childhood SES and increased risk of exposure to cardiovascular disease.
- Adolescents with a higher parental education do have a lower WBCs count and proportion of neutrophils.

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