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Normal levels of soluble E-selectin, soluble intercellular adhesion molecule-1 (sICAM-1), and soluble vascular cell adhesion molecule-1 (sVCAM-1) decrease with age

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SUMMARY

sE-selectin, sICAM-1, sVCAM-1 and von Willebrand factor (vWF) were assayed in 238 samples in a longitudinal study of 81 normal children from 9·5 to 15·5 years old. Multilevel modelling was used to quantify changes with age. sE-selectin, sICAM-1 and sVCAM-1 all fell significantly over the age range (by 17%, 16%, and 10%, respectively). In contrast, levels of vWF were not age-dependent. Our findings highlight the need for age-matched controls when studying cell surface adhesion molecules in disease groups, and may imply developmental changes in expression of these molecules and their shedding from the cell surface.

Keywords children age factors cell adhesion molecules von Willebrand Factor

INTRODUCTION

Adhesion molecules on the cell surface, including E-selectin, ICAM-1, and VCAM-1, play an important role in cell–cell interaction [1,2]. Elevated levels of soluble forms of these molecules are found in a variety of inflammatory conditions [3]. While levels in healthy adults are widely reported [3] and available from the manufacturers of commercial kits, normal ranges for children have not been established. We have previously found levels of these three circulating cell adhesion molecules to be higher in normal children (median age 5 years) than in adults [4]. The aim of this study was to confirm, in a larger group of older children studied longitudinally, that normal levels of sE-selectin, sICAM-1 and sVCAM-1 are age-related. Circulating von Willebrand factor (vWF) was also measured.

SUBJECTS AND METHODS

Subjects

A total of 238 serum samples was studied, taken from 81 healthy children (40 male) living in the west of England. Samples were taken as part of a prospective longitudinal study of growth and endocrine changes, reported in detail elsewhere [5,6]. The children were seen at regular intervals between 9 and 16 years. We studied samples taken on four occasions at 2 year intervals, when the children were aged 9–10 (n = 64), 11–12 (n = 63), 13–14 (n = 57), and 15–16 years (n = 54). Mean ages were 9·5, 11·5, 13·5 and 15·5 years. Samples were available from 31 of the children at all four ages, 27 at three ages, 10 at two ages and from the remaining 13 children once. All were normal children who were well enough to attend school on the day the blood was taken. Blood was taken in the late morning, and stored at –20°C until analysed.

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Assay methods

Soluble cell adhesion molecules were measured by sandwich ELISA using commercial kits from R&D Systems (Abingdon, UK; previously British Biotechnology). In brief, microtitre wells coated with anti-human sE-selectin, sICAM-1 or sVCAM-1 were incubated with diluted samples, standards and controls, before incubation with conjugated anti-human cell adhesion molecules and substrate. vWF antigen was assayed by sandwich ELISA based on the method of Short *et al.* [7] as previously described [4]. Plates were coated with rabbit anti-human vWF antiserum (Dako, High Wycombe, UK) and incubated with diluted samples followed by peroxidase-conjugated rabbit anti-human vWF (Dako) and substrate. Results were calculated from a curve generated from a national standard (NIBSC, Potters Bar, UK) and are reported as percentage of pooled normal plasma, with 100% equivalent to 0·88 IU.

Assays were run blind with each age group tested in equal numbers on each plate. Serial samples from a given child were run together on the same plate. The study was approved by the ICH Ethical Committee.

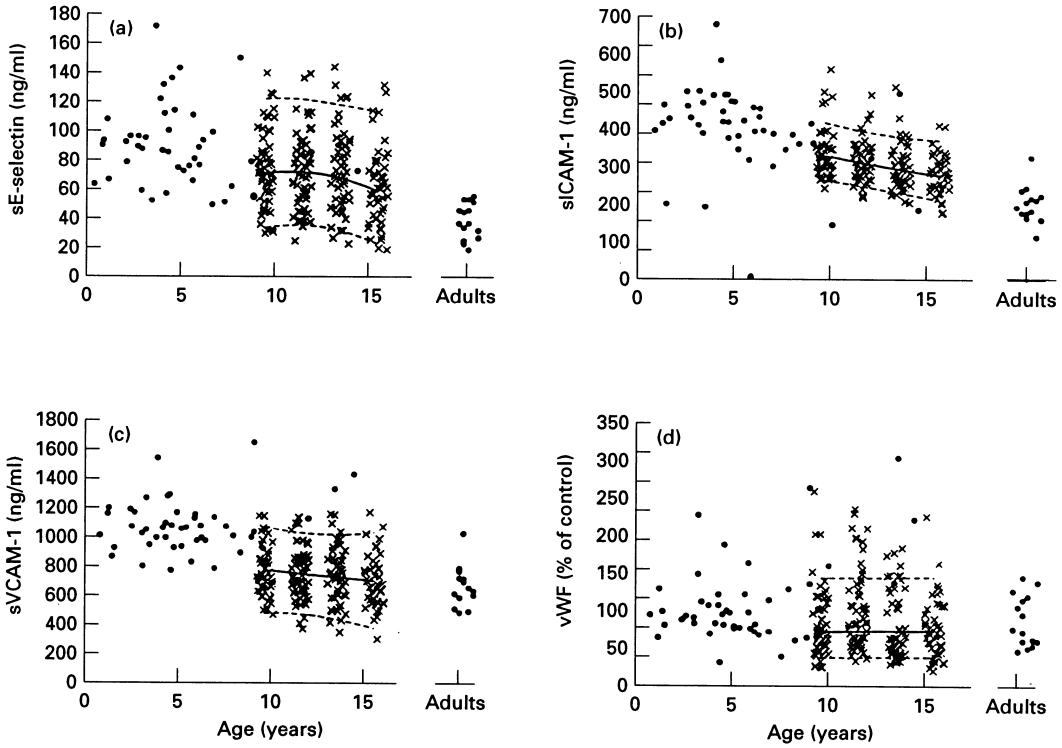

Fig. 1. Values of sE-selectin, sICAM-1, sVCAM-1(ng/ml), and von Willebrand factor (vWF) (% of normal control) against age in years. ×, Data from the current study; ●, data from young children and adults as previously reported [4]. Solid lines represent the average change with age. Dotted lines indicate the range within which 95% of children's curves are expected to lie. Note that this is not a reference range for single observations.

Statistical analysis

Paired *t*-tests were used to compare values at 9·5 and 15·5 years in those children with data available from both ages. Results of this exploratory analysis are shown with 95% confidence intervals (CI). Because there were variable numbers of observations for individual children, multilevel modelling was used in order to allow the use of all available data [8,9]. For each of the four assayed variables, summary curves were determined across the age range, together with the extent to which an individual would be expected to deviate from these. In addition, a range was calculated that would be expected to contain the average progression with age of 95% of children. Where necessary, transformations to normality were performed before analysis.

RESULTS

There were 37 children with measurements available at 9·5 years and 15·5 years. Values of sE-selectin, sICAM-1 and sVCAM-1 fell significantly over this period ($P < 0{\cdot}001$ for all three). The mean decreases (95% CI) were 17 ng/ml (10, 24), 59 ng/ml (39, 79) and 106 ng/ml (46, 166), respectively. VWF did not change significantly ($P = 0{\cdot}67$), with a mean decrease of 2% (–9, 13).

For the multilevel modelling, sE-selectin, sICAM-1, and vWF required transformation to induce normality. Soluble VCAM-1 was normally distributed, with one outlying value of 2000 ng/ml at age 15·5 years. No reason could be found to explain this outlying value. Conclusions regarding changes in sVCAM-1 with age were unaltered whether the data were analysed with or without this value, although the variability of the predicted curve was affected. Results are presented after its exclusion. The data are shown together with the best fitting models, and the range within which 95% of children's curves are expected to lie (Fig. 1). Note that this does not represent a reference range for single observations. For vWF there was a relatively high within-child variability, illustrated by the large number of points outside the 95% range (Fig. 1d).

Previously reported values [4] from 48 healthy children (median 5 years) and 15 healthy adults (median 32 years) are also shown (Fig. 1). These results, with higher levels in young children and lower in adults, are consistent with the current study. However, sVCAM-1 levels in 9–16-year-olds are lower than would be predicted by extrapolation of the earlier data (Fig. 1c).

DISCUSSION

E-selectin (CD62E, ELAM-1), ICAM-1 (CD54) and VCAM-1 (CD106) are cell surface adhesion molecules on vascular endothelial cells. They are up-regulated by inflammatory cytokines and regulate the adhesion and migration of leucocytes across the endothelium. ICAM-1 and VCAM-1 are also found on a variety of other cell types [1,2]. Circulating adhesion molecules are probably formed by cleavage and release into the circulation of the extracellular domain of the membrane-bound form [10–12]. Elevated levels of circulating adhesion molecules have been reported in numerous disease states, including infections, systemic vasculitis, neoplasia and after transplantation [3].

Studies in adults suggest that levels of sE-selectin, sICAM-1, and sVCAM-1 are constant with age between 18 and 65 years, although few details are given [10,12–14]. A recent study using an

in-house immunoassay reported higher levels of sICAM-1 in 10 normal pre-school children (mean age 2 years) than in 10 school age children (mean age 8 years) [15]. In another study, values of these three soluble cell adhesion molecules in 48 healthy children (median age 5 years) were found to be approximately double those in adults [4]. We have now confirmed that levels of sE-selectin, sICAM-1 and sVCAM-1 are age-related, falling significantly between 9 and 16 years.

This study was longitudinal, and samples from different age groups were by necessity stored for different lengths of time before analysis. This could be a source of artefactual age-related changes. However, any storage-related loss of detectable cell adhesion molecules would be greater in samples stored for longer periods, i.e. those taken when the children were younger. Conversely, this would be least in the samples from the oldest children. Thus, deterioration of the samples with prolonged storage would lead to an apparent increase in levels with age, rather than the observed decrease. We measured levels of serum vWF, an adhesive glycoprotein derived mainly from endothelial cells [16], in the same samples. There was no significant change in vWF levels with age, consistent with previous work [17]. In addition, the previously reported differences in levels of cell adhesion molecules between young children and adults were found in samples stored for short and equal periods at –70°C [4]. Samples in the current study had already been subjected to a number of freeze–thaw cycles, and levels of sICAM-1 have been reported to increase by a mean of 13 ng/ml after eight freeze–thaw cycles [4]. However, this is less than the difference seen in this study. There was no change in levels of sE-selectin, sVCAM-1, or vWF after multiple freeze–thaw cycles [4]. Thus our results appear not to be an artefact of prolonged storage or multiple freeze–thaw cycles.

In this report we studied children between 9 and 16 years old, and our results cannot be reliably extrapolated to younger children or adults. The numbers studied are sufficient to demonstrate changes with age, but not to provide reliable age-related normal ranges [18]. However, it can be seen from Fig. 1 that results from a wide age range appear consistent with a decrease from early childhood to young adulthood. There is good congruence between the current data and what we previously reported for sE-selectin, sICAM-1, and vWF, but levels of sVCAM-1 in the current study are lower than would be predicted by extrapolation of the earlier data (Fig. 1c). This may be a true result, or reflect loss of sVCAM-1 activity with storage. Alternatively, the explanation may be interassay variation, as the assays for the two studies were run separately, and the interassay CV quoted for the sVCAM-1 kit is 9–10% (R&D Systems). This would not affect the conclusions of the present study.

Maturational changes in the immune system are well recognized. Recently levels of L-selectin, both on neutrophils and in the soluble form, have been reported to be age-related [19]. The function *in vivo* of soluble cell adhesion molecules has not been established, and levels measured by immunoassay in serum may not reflect biological activity, or concentrations in the microenvironment. The physiological importance of changes with age in levels of soluble cell adhesion molecules is thus unknown.

In conclusion, we have shown that levels of sE-selectin, sICAM-1, and sVCAM-1 are age-related, with a significant decrease between 9 and 16 years. Age-appropriate control groups are essential in any study of these molecules in children. Larger numbers are needed to produce accurate and reliable age-related standards. It is clear that there is a wide range of values in normal individuals, and thus direct comparisons between 'patients' and 'normals' must be interpreted with caution. The functional significance of the age-related decrease in sE-selectin, sICAM-1, and sVCAM-1 is unknown.

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REFERENCES

1 Springer TA. Adhesion receptors of the immune system. Nature 1990; **346**:425–3.
2 Carlos TM, Harlan JM. Leukocyte-endothelial adhesion molecules. Blood 1994; **84**:2068–101.
3 Gearing AJ, Newman W. Circulating adhesion molecules in disease. Immunol Today 1993; **14**:506–12.
4 Nash MC, Shah V, Dillon MJ. Soluble cell adhesion molecules and von Willebrand factor in children with Kawasaki disease. Clin Exp Immunol 1995; **101**:13–17.
5 Preece MA, Cameron N, Donmall MC *et al.* The endocrinology of male puberty. In: Borms J, Hauspie R, Sands A, Suzanne C, Hebbelink M, eds. Human growth and development. New York: Plenum Publishing Corporation, 1984: 23–37.
6 Dunger DB, Perkins JA, Jowett TP *et al.* A longitudinal study of total and free thyroid hormones and thyroxine binding globulin during normal puberty. Acta Endocrinol Copenh 1990; **123**:305–10.
7 Short PE, Williams CE, Picken AM, Hill FGH. Factor VIII related antigen: an improved enzyme immunoassay. Med Lab Sci 1982; **39**:351–5.
8 Goldstein H. Efficient statistical modelling of longitudinal data. Annals of Human Biology 1986; **13**:129–41.
9 Woodhouse G, ed. A guide to ML3 for new users, 2nd edn. London: Institute of Education, University of London, 1993.
10 Newman W, Beall LD, Carson CW *et al.* Soluble E-selectin is found in supernatants of activated endothelial cells and is elevated in the serum of patients with septic shock. J Immunol 1993; **150**:644–54.
11 Rothlein R, Mainolfi EA, Czajkowski M, Marlin SD. A form of circulating ICAM-1 in human serum. J Immunol 1991; **147**:3788–93.
12 Wellicome SM, Kapahi P, Mason JC, Lebranchu Y, Yarwood H, Haskard DO. Detection of a circulating form of vascular cell adhesion molecule-1: raised levels in rheumatoid arthritis and systemic lupus erythematosus. Clin Exp Immunol 1993; **92**:412–8.
13 Stegeman CA, Tervaert JW, Huitema MG, de Jong PE, Kallenberg CG. Serum levels of soluble adhesion molecules intercellular adhesion molecule 1, vascular cell adhesion molecule 1, and E-selectin in patients with Wegener's granulomatosis. Relationship to disease activity and relevance during followup. Arthritis Rheum 1994; **37**:1228–35.
14 Mason JC, Kapahi P, Haskard DO. Detection of increased levels of circulating intercellular adhesion molecule 1 in some patients with rheumatoid arthritis but not in patients with systemic lupus erythematosus. Lack of correlation with levels of circulating vascular cell adhesion molecule 1. Arthritis Rheum 1993; **36**:519–27.
15 Furukawa S, Imai K, Matsubara T *et al.* Increased levels of circulating intercellular adhesion molecule 1 in Kawasaki disease. Arthritis Rheum 1992; **35**:672–7.

16 Blann A. von Willebrand factor and the endothelium in vascular disease. Br J Biomed Sci 1993; **50**:125–34.
17 Andrew M, Vegh P, Johnston M, Bowker J, Ofosu F, Mitchell L. Maturation of the hemostatic system during childhood. Blood 1992; **80**:1998–2005.
18 Wade AM, Ades AE. Age-related reference ranges: significance tests for models and confidence intervals for centiles. Stat Med 1994; **13**:2359–67.
19 Rebuck N, Gibson A, Finn A. Neutrophil adhesion molecules in term and premature infants: normal or enhanced leucocyte integrins but defective L-selectin expression and shedding. Clin Exp Immunol 1995; **101**:183–9.