

Variations in serum soluble IL-2 receptor concentration

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Soluble interleukin-2 receptor (sIL-2R) concentrations were measured in the sera of 18 healthy adult volunteers, 40 healthy children aged 5 months – 16 years 8 months and cord blood from 10 babies, using two commercially available ELISA kits (T-cell Sciences and Serotec). In the presence of normal C-reactive protein (CRP) children (aged 0–16 years 8 months) had significantly higher sIL-2R concentrations than adults aged (18–37 years). In children up to the age of 16 years there was a significant negative correlation between age and sIL-2R ($r = -0.725$ $p < 0.0001$ T-Cell Sciences, $r = -0.646$ $p < 0.0001$ Serotec). A comparison was also made between the two kits, which indicated that both kits yielded reproducible results but the T-Cell Sciences kit had a larger working concentration range than the Serotec kit. Following 12-h sampling in adult volunteers, a lower concentration of sIL-2R was observed at 4 am compared with 10 am ($p = 0.0052$) which indicates diurnal variation. These observations emphasize the importance of the study of normals as part of any research, and raises further questions regarding the many factors which influence immune function.

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There are many intercellular mediators (cytokines) involved in the development of specific immune responses (1–3). Interleukin-2 (IL-2) and the expression of high-affinity receptors for IL-2 (CD25) have been shown to be vital for the growth and proliferation of T cells (4), which play a pivotal role in switching on the immune response. Furthermore IL-2 receptors have also been demonstrated on activated B-lymphocytes (5, 6), natural killer cells (7), eosinophils (8), alveolar macrophages and monocytes (9), and mast cells and basophils (10). In addition to cell-surface receptors for IL-2 it has been shown that following activation, T cells release a soluble form of receptor (sIL-2R) which can be readily measured in serum (11). With its implication as a marker of cellular immune system activation, elevated sIL-2R levels have been found in many conditions, including auto-immune disorders (12), certain lymphoid neoplasms (13), and those suffering graft rejection following transplant surgery (14). It has also been shown that normal individuals have measurable concentrations of sIL-2R in their serum.

With all the current interest in sIL-2R levels in various pathological conditions it is at least as important to characterize the physiological variation of

this marker. Our aim in this study was to establish a normal range for sIL-2R in cord blood and in children, to compare this with adult values, and to examine the pattern of sIL-2R variation over 12 h.

These results were obtained using two commercially available ELISA kits (T-Cell Sciences and Serotec). These kits were chosen since they are two ends of the spectrum regarding price, and they report completely different concentrations of sIL-2R, units/ml for T-Cell Sciences and ng/ml for Serotec.

Subjects & methods

Cord blood samples were obtained from the umbilical cord at the delivery of normal full term babies delivered at the Princess Anne Hospital, Southampton. The paediatric group was drawn from children attending the Paediatric Day Surgery Unit at Southampton General Hospital for routine surgical procedures, including hernia and hydrocoele repairs, circumcisions, and dental extractions. Children with any history of asthma/eczema/hay fever, any known allergies, or any recent infections were excluded. Written consent for inclusion in the study was obtained from the child's parent(s)/guardian. Venous

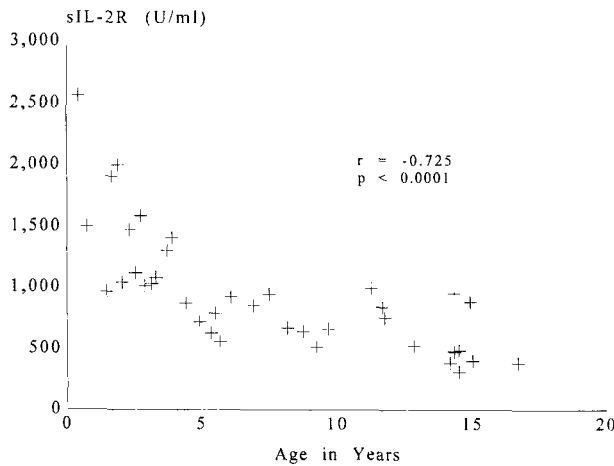


Fig. 1. Scatter plot of sIL-2R concentrations of 39 healthy children aged between 5 months and 16 years 8 months showing a significant negative correlation using the T-Cell Sciences kit. Similar results were obtained using the Serotec kit ($r = -0.646$, $p < 0.0001$).

blood samples were taken from these children following induction of general anaesthesia, and prior to surgery.

Serum samples from the non asthmatic normal adult group were taken prior to entry into a study of the side effects of two inhaled corticosteroid preparations. Participants were similarly screened for asthma or atopic diseases, and excluded if they had recently used topical or systemic corticosteroids. As part of their assessment individuals had an intravenous cannula inserted, and small blood samples removed at intervals throughout a 12 hour period (approx. 10 pm–10 am). In this study 3 serum samples were analysed from each adult: an evening sample, and an early and late morning sample, corresponding approximately to 10 pm, 4 am and 10 am.

C-reactive protein (CRP) levels were measured on all paediatric subjects in order to rule out the presence of infection. Measurements were made using an ELISA technique developed by the Southampton Tenovus Research Laboratories. Using conventional methods of analysis a CRP level of >6.0 mg/l is usually taken as indicative of infection. However on Tenovus recommendations regarding CRP levels obtained using the more sensitive ELISA technique, any child having CRP >2.4 mg/l was excluded. Since these children were attending hospital for surgery the presence of other infections (e.g. viral) was unlikely.

Blood/serum samples

In all cases blood was allowed to clot, centrifuged at 2,500 RPM for 10 min, and the serum fraction removed and stored at -30°C until analysis. In 18 cases blood samples were also taken into hepari-

nised vacutainers and differential white blood cell counts performed.

sIL-2R ELISA kits

Soluble IL-2R measurement was performed using a CELLFREE ELISA Test Kit (T Cell Sciences, Inc. Cambridge, MA, USA), and a Serotec sIL-2R ELISA kit (Serotec, Oxford, UK). Coefficients of variation were quoted by both manufacturers for intra-assay and inter-assay variation (T-Cell Sciences, 4.6–8.7% and 7.1–9.4%, Serotec, 6% and 9% respectively).

Comparisons were made between the two kits on samples assayed on the same occasion and between the same kit on samples assayed on two separate occasions.

Statistics

Statistical analyses were by ARCUS computer program using Mann-Whitney U test, Wilcoxon rank paired test and Spearman correlation analysis, a p value of <0.05 was considered significant. The reproducibility of results from each kit was demonstrated using analysis as described by Bland & Altman (15). This study was approved by the Southampton Joint Ethics Sub Committee.

Table 1. Soluble IL-2R levels in healthy adults and children grouped according to age (measured using T-Cell Sciences and Serotec ELISA kits). Values in the 8y–11y 9m age group are shown (i) with and (ii) without the statistically outlying point (1357 U/ml T-Cell Sciences, 5.7 ng/ml Serotec). Significantly higher concentrations of sIL-2R were found in all children aged <16 y 8m compared to adult values. Cord ($p=0.02$), 5m–3y 11m ($p<0.0001$), 4y–7y 6m ($p<0.0001$), 8y–11y 9m ($p<0.0001$), 12y–16y 8m ($p<0.006$). Similar statistical significances were obtained with the Serotec kit.

Age group	Number	T-cell sciences		Serotec	
		Range ng/ml (median)	Standard dev.	Range U/ml (median)	Standard dev.
CORD	n = 10	212–1153 (572)	285	0.45–2.9 (1.7)	0.8
5m–3y11m	n = 14	965–2570 (1355)	466	3.1–9.5 (5.4)	1.6
4y–7y6m	n = 8	558–943 (820)	139	1.8–3.8 (3.5)	0.8
8y–11y9m (i)	n = 8	514–1357 (713)	265	1.7–5.7 (2.7)	1.4
(ii)	n = 7	514–993 (671)	155	1.7–4.8 (2.7)	1.0
12y–16y8m	n = 10	309–950 (478)	216	0.8–5.9 (1.4)	1.6
ADULTS	n = 15	83–568 (342)	141	0.0–2.6 (0.7)	0.7

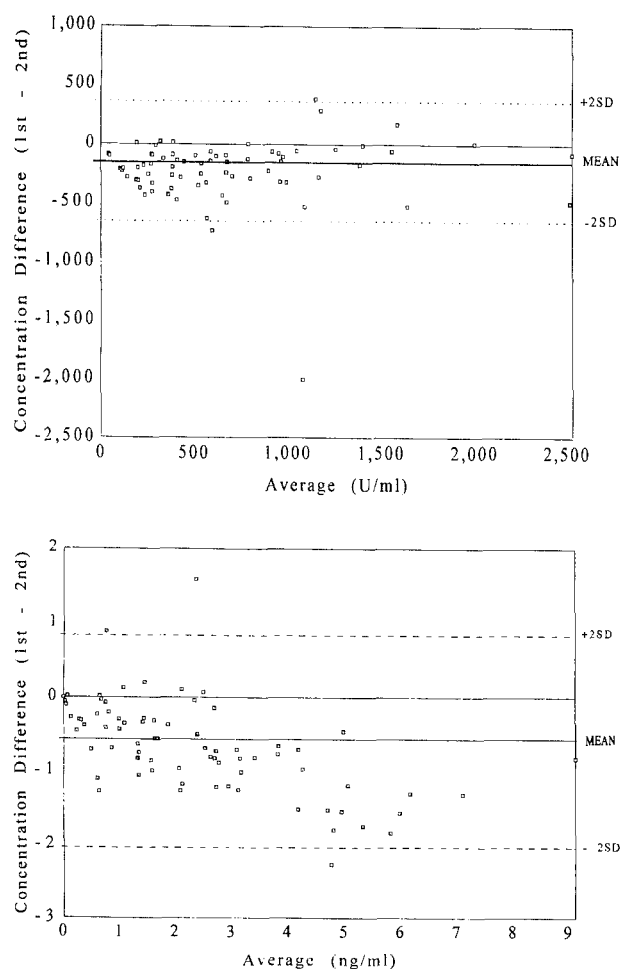


Fig. 2a, b. Bland and Altman analysis performed on 69 samples assayed using a) T cell Sciences ELISA kits and b) Serotec ELISA kit.

Results

The sIL-2R concentration for the cord blood was over the range of 212–1153 U/ml (median 572 U/ml) for the T-cell Sciences kit, and 0.45–2.9 ng/ml (median 1.7 ng/ml) for the Serotec kit.

The age range of the paediatric group was 5 months – 16 years 8 months (mean 7yrs 3m), and the mean (SD) CRP level was 0.3 (0.47) mg/l. Plotting individual concentrations showed there to be a significant negative correlation between age and sIL-2R ($r = -0.725$, $p < 0.0001$, T-Cell Sciences (Fig. 1) and similarly, $r = -0.646$, $p < 0.0001$, Serotec).

The age range of the adult group was 18–37 years (mean 25 yrs 7 m). Using the T-Cell Sciences kit the mean adult sIL-2R level was 363 U/ml. This is lower (although it is within the normal range) than the T-Cell Sciences published figures from a group of 42 healthy blood donors; mean sIL-2R 529 U/ml, SD 192 U/ml. Using the Serotec kit the mean adult concentration was 0.8 ng/ml. This was within the normal range published by Serotec; mean 0.69

ng/ml, range 0.03–6.4 ng/ml ($n = 138$). For comparison of paediatric and adult groups children were divided into 4 groups according to age, Table 1 (T-Cell Sciences and Serotec). Children aged 5 months – 3yrs 11 months had significantly higher sIL-2R levels than adults ($p < 0.0001$), and indeed higher than individuals in any other group. Two sets of results are shown for the age group 8 years – 11 years 8 months since one subject was found to have a sIL-2R concentration of 1357 U/ml (T-Cell Sciences). This value was considered an outlier for this age group following discussion with the Medical Statistics Department of Southampton University and is, therefore, removed to report the normal range, although its inclusion did not affect the statistical significance. Results have been expressed as median and range for both the T-Cell Sciences and the Serotec kits. All children aged < 16 years 8 months had significantly higher concentrations of sIL-2R than adults when either kit was used.

A comparison was made between sIL-2R concentrations and lymphocyte numbers on a subgroup ($n = 18$) of the normal children using the T-Cell Sciences kit. There was no correlation between lymphocyte count and sIL-2R concentration ($r = 0.443$, $p > 0.2$).

Bland & Altman analysis on data from the two kits performed on two occasions indicated good reproducibility with only 4% of points falling outside 2 standard deviations of the mean difference for both the T Cell Sciences (Fig. 2a) and Serotec (Fig. 2b) ELISA kits. However, at high concentrations of sIL-2R the Serotec kit became less reliable and the differences became increasing large when the concentration exceeded 4 ng/ml. There was good correlation between the same samples assayed using the two types of kit $p < 0.0001$ (data not shown).

Using a Wilcoxon Rank paired test to compare sIL-2R levels at different times in individual adults revealed that 4am sIL-2R levels were significantly lower than 10 am levels ($p = 0.0052$), Fig. 3.

Discussion

The study of normal individuals is a vital aspect of any research whenever a new test becomes available, since it is only after this that one can judge what is abnormal. When defining the normal range for sIL-2R concentration as an indicator of immune system activity, it is important to consider how it varies with age.

The possibility that children might have higher levels of sIL-2R than adults was first suggested by Pui et al (16), who measured serum sIL-2R in 12 children with otitis media, who were otherwise well, and aged from 1–6 years. On finding elevated levels in these children, compared to healthy adults, they

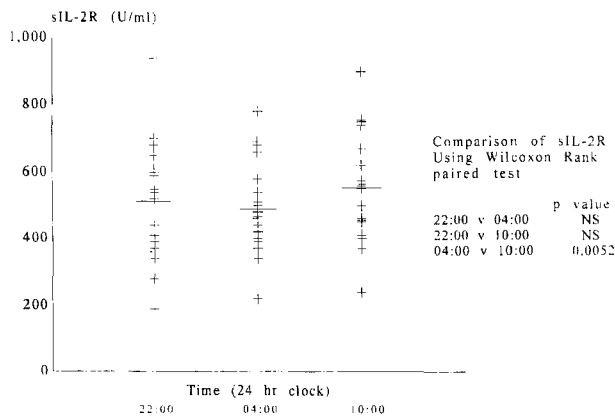


Fig. 3. Diurnal variation of sIL-2R in 18 healthy adults showing individual points and mean concentrations of sIL-2R measured using a T-Cell Sciences kit.

speculated that this was probably the result of lymphocyte activation in response to infection.

Subsequently in correspondence to the editor of Blood, Komp et al., 1988 (18) reported results of serum sIL-2R analysis in 122 normal uninfected children. Even in the absence of infection, they too found that children, in particular those aged 9.5–19.5 months, had markedly elevated sIL-2R compared to adult normals.

In our study it has been demonstrated that normal non-asthmatic, non-allergic, uninfected children aged between 5 months – 3 years 11 months had significantly higher sIL-2R levels than individuals of any other age. A possible hypothesis for this is that children aged 5 months – 3 years 11 months have higher lymphocyte counts and consequently more sIL-2R in serum than older children. However although there was a trend, there was no significant correlation when comparing lymphocyte counts with sIL-2R concentrations. This suggests that higher sIL-2R concentrations may not simply be reflecting the presence of higher lymphocyte numbers.

Another possible explanation lies in the vast immunological challenge, and the constant exposure to new antigens, during the first few years of life. High levels of leucocyte activation may be related to transient immune reactions to dietary antigens and bacteria in the small intestine (17).

Results obtained using the two ELISA kits showed good correlation between the assay techniques. The adult values observed were within the normal range quoted for each kit and the overall trend relating age to concentration was similar for both kits. Interestingly however the mean normal adult value obtained using the T Cell Sciences kit, mean 363 U/ml was lower than published. The 42 blood donors tested and quoted in their literature were only described as healthy, and could have higher levels of sIL-2R due to other allergic prob-

lems, eg rhinitis, whereas those in this study were allergy skin prick test negative.

The results obtained from the two kits are expressed in different units, Units/ml for the T Cell Sciences kit and ng/ml for the Serotec kit. There is a conversion factor available for the T Cell Sciences assay to convert Units/ml into ng/ml ($\text{Units/ml} \times 0.113 = \text{ng/ml}$). When this is performed on the normal adult value quoted (529 U/ml) then this leads to an absolute value of 59.8 ng/ml which is very different to the value quoted by Serotec (0.69 ng/ml). Although the values obtained for each kit are quite different to each other, neither are wrong and it is a personal preference as to which kit to choose. As long as the same kit is used throughout the entire investigation it should be possible to interpret the results as long as the normal range is available.

There are differences in the standard curve ranges for the 2 kits, T Cell Sciences ranges from 0–6650 U/ml and Serotec from 0–8 ng/ml. The maximum value for any normal child in the group investigated was 2570 U/ml and >8 ng/ml, for the T Cell Sciences and Serotec kits respectively. Hence using the Serotec kit the possibility arises of the concentration for a normal child being higher than the most concentrated standard. Consequently a child with a raised sIL-2R concentration (eg asthma, arthritis) would certainly be beyond the scope of the standard curve, and the serum sample would need to be diluted. Since the scope of the T Cell Sciences standard curve is much greater, then the question of dilution is less likely to arise.

There was good reproducibility between samples assayed using the same kit and the same samples on two separate occasions, shown by Bland & Altman analysis. It is noticeable that the mean of the differences determined by this analysis for both of the ELISA kits are less than the expected value of zero. A possible explanation for this is that the ambient temperature on the second occasion of analysis was higher than on the first occasion. This could have effected the incubations carried out at room temperature leading to different final values. This is important to consider when it is not possible to perform all the analyses on the same day.

The Bland and Altman analysis also highlighted a trend when using the Serotec kit of larger differences as the concentration of the sample exceeds 4 ng/ml. This corresponds to only half of the standard curve range and in order to make this kit as accurate as possible it may be necessary to dilute samples to <4 ng/ml.

What is of additional note from the adult data is the presence of significantly lower sIL-2R levels at 4am than 10 am ($p < 0.01$). The mean and range of levels in both groups is essentially very similar and such variation has never been shown elsewhere.

Hormonal influences may be responsible although mechanisms of action remain unclear. Hormones with fairly well characterised diurnal rhythms are growth hormone (GH) and the glucocorticoids, e.g. cortisol. However, with 4am sIL-2R levels significantly lower than 10 am levels, it seems unlikely that normal fluctuations in GH and cortisol could account for this difference.

Could subtle variations in other adrenal hormones play a part in immune modulation? For example, lymphocytes are known to possess cell-surface beta-adrenoceptors, and fluctuations in circulating levels of catecholamine might play some role in influencing leucocyte function. Such an effect would depend on the number and sensitivity of beta-adrenoceptors, and the degree of catecholamine variation, neither of which are known. While it seems unlikely that there is any physiological importance to the observed variation, it may be important to consider when comparing samples taken from individuals at different times.

In this communication we have reported the normal range of sIL-2R concentrations with particular emphasis on childhood values which are significantly raised in comparison to adult concentrations.

The two kits we have tested are comparable and reliable, but it is important to use the same type of kit throughout the entire investigation and to ensure that a normal range is available. It is also important to monitor the ambient temperature.

In addition some consideration must be given to the timing of the sampling since subtle, but significant differences arise at different times of the day.

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