In Vivo Production of Type 1 Cytokines in Healthy Sickle Cell Disease Patients

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Interleukins (IL)-1, 2, 12, and interferon (IFN)-γ, along with soluble IL-2 receptor (sIL-2R) were measured from sera obtained from healthy sickle cell disease (SCD) patients and comparable healthy control subjects. The cytokines were assessed by enzyme-linked immunosorbent assay (ELISA) in 60 SCD patients and 58 controls. No significant detectable levels of IL-1 or IL-12 were found in the sera of either group of patients. Significantly elevated levels of IFN-γ were measured in 20 (33%) of 60 SCD patients and 21 (36%) of 58 controls. A large subset of 18 (41%) of 43 healthy controls and a smaller subset of 12 (21%) of 58 SCD demonstrated detectable levels of IL-2. The sIL-2R levels of the SCD group (4465±552 pg/mL) were significantly higher ($P<.0001$) than that of controls (3473±411 pg/mL). The results revealed comparable circulating levels of all type 1 cytokines in both healthy SCD and normal control subjects, with the exception of in vivo sIL-2R production. Elevated serum levels of both IL-6 and tumor necrosis factor (TNF)-α have been reported previously in a significant percentage of SCD steady-state subjects. These two cytokines are known to increase sIL-2R expression and may help explain the difference between the patient populations. Immune activation markers such as sIL-2R are produced by cells that mediate host responses to infection or inflammatory stimuli. The implication of higher levels of sIL-2R in SCD is not clear, but chronic parvovirus B19 infection, chronic polyclonal activation of B cells, or defective regulation of antibodies are possible explanations for the elevated levels in SCD. (*J Natl Med Assoc.* 1999;91:619-624.)

Key words: sickle cell disease ◆ cytokines ◆ receptors

Sickle cell disease (SCD) in children may be characterized by significant morbidity and early mortality in the natural course of disease.[1-3] Children with this hemoglobinopathy exhibit many of the clinical manifestations associated with immunodeficiency disorders, as evidenced by the high predilection for frequent and severe bacterial infections.[4-6] Multiple derangements of immune function have been chronicled in the literature to explain these phenomena, including dysfunction of the spleen, humoral response, opsonophagocytosis, and the alternate pathway of complement.[7-10] In recent years, more data have been reported on the status of cell-mediated immunity (CMI) in SCD.[11-20] This study evaluates in vivo production of type 1 cytokines during the steady state of SCD patients as compared to normal healthy controls.

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MATERIALS AND METHODS

Study Population

Sixty SCD patients in the steady state of disease

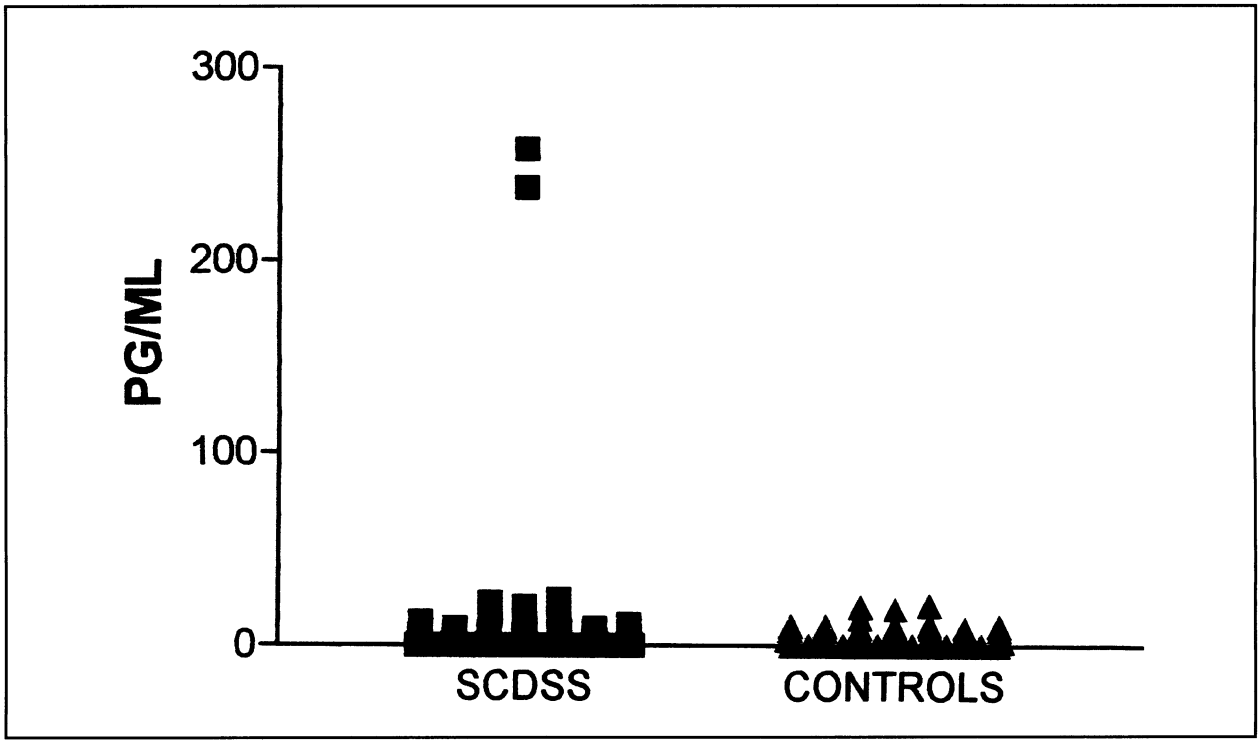

Figure 1. Individual IFN-γ serum level determinations were done by ELISA technique, as described in Materials and Methods. Elevated levels (>5 pg/mL were found in 18 (30%) of 60 SCD patients (range 6-258 pg/mL) and 21 (36%) of 58% controls (range: 8-21 pg/mL).

comprised the study population. Patient ages ranged from 1 to 17 years (32 subjects) and 18 to 45 years (28 subjects). Fifty-six of the SCD patients had sickle cell anemia, 2 patients had sickle cell-hemoglobin C disease, and 2 patients had sickle cell-thalassemia disease. All of the SCD patients underwent follow-up at the King/Drew Medical Center and were crisis-free for a least one month prior to entrance into the study.

Fifty-eight patients comprised the control group and were matched for age with 35 subjects in the 1 to 17-year age group and 23 in the older age group. In addition, the groups were matched for gender. All of the SCD patients were black, whereas the control group contained 65% black and 35% Hispanic patients. The study was approved by the Committee for the Protection of Human Rights (institutional review board). Informed consent was obtained from parents or guardians of subjects 1-17 years of age and directly from individuals ⩾18 years.

Serum Acquisition

Nonheparinized blood was obtained from the antecubital vein in both SCD and healthy normal control subjects. Serum was then separated by centrifugation, aliquotted, and stored at −20°C for later assays.

Interleukin Assays

Human IL-1, IL-2, sIL-2R, and IFN-γ enzyme-linked immunosorbent assay (ELISA) kits (Endogen, Cambridge, MA) were used to measure serum levels. The kits were supplied with precoated plates, and standards were reconstituted as directed after adding a plate reagent to each wel. Fifty microliters of standards or sera in duplicate were added, and the plate was incubated for one hour at 25°C. Unbound material was then removed by aspiration and washing. The next conjugate reagent was added, and the plate was incubated for 30 minutes. After incubation, unlabeled antibody in the conjugate reagent was removed by aspiration and washing. The bound IL-1, IL-2, sIL-2R, and IFN-γ were calculated by color change reaction using an ELISA reader (Titertek Multiskan, Flow Laboratories Inc, McLean, VA) set at 450 nm.

The quantitation of IL-12 entailed similar methodology, but 200-μL samples were required and the ELISA kit was manufactured by R&D Systems Inc (Minneapolis,

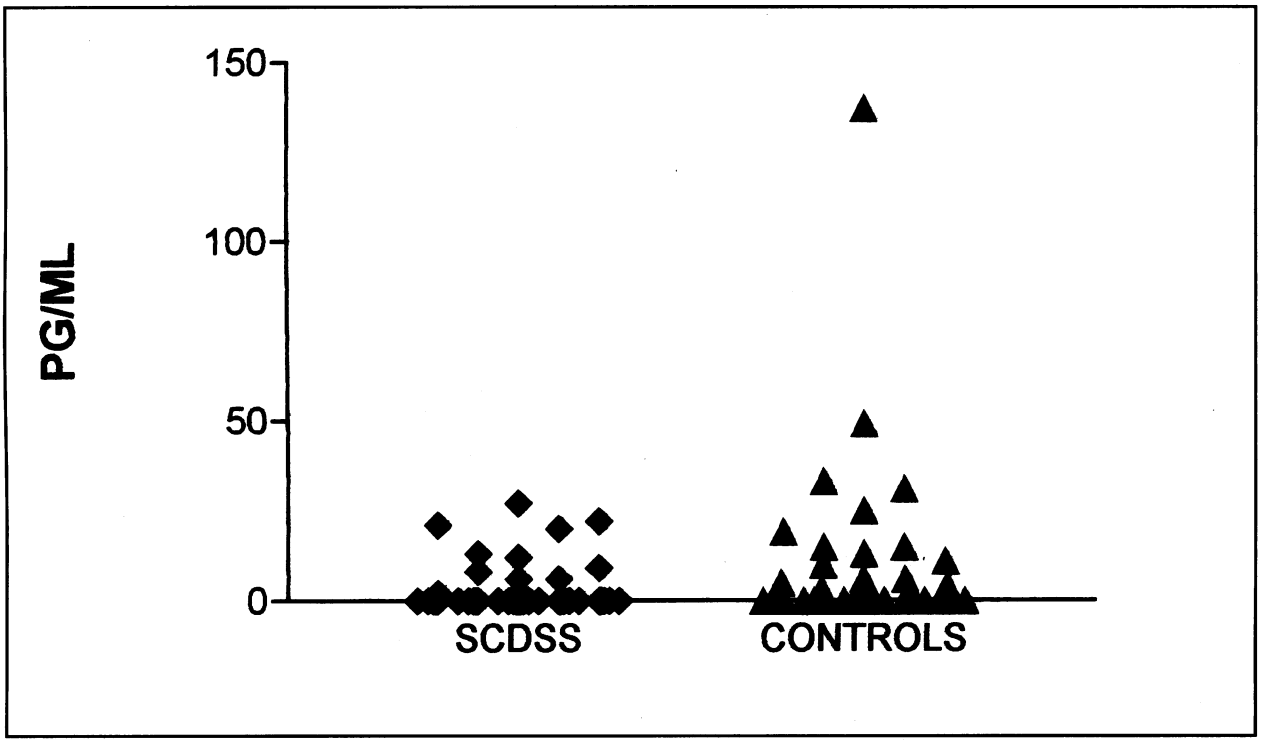

Figure 2. Individual IL-2 serum determinations were done by ELISA technique, as described in Materials and Methods. Detectable levels were present in 12 (21%) of 58 SCD patients (range: 2-27 pg/mL) and 18 (41%) of 43 controls (range: 1-137 pg/mL.)

MN). Concentrations of individual samples were determined by referring to the standard curve and expressed as pg/mL. Normal ranges of serum cytokine levels were provided by each vendor. The sensitivity of the ELISA kits are: IL-1 <2 pg/mL, IL-2 <6 pg/mL, sIL-2R <212 pg/mL, and IL-12 <5 pg/mL. The intra-assay and inter-assay CV were <10% in all kits used in this study.

Statistical Analysis

Concentrations are expressed as mean titers ± standard error of the mean for comparison of levels of sIL-2R in SCD and normal control patients. Statistical analysis was done using a paired *t* test.

RESULTS

No detectable levels of IL-12 were found in 43 SCD steady-state patients or 27 healthy normal control subjects. Insignificant levels of IL-1 were detected in 12 (36%) of 33 SCD subjects (range: 1-5 pg/mL) and 3 (11%) of 28 controls (all detectable at 1 pg/mL). Elevated levels of IFN-γ (>5 pg/mL) were detected in 18 (30%) of 60 SCD patients (range: 6-258 pg/mL) and 21 (36%) of 58 control (range: 8-21 pg/mL). Two (3%) of the 60 SCD subjects had extremely high levels of IFN-γ (238 and 258 pg/mL), but the rest were in the same range as the healthy control subjects. These results are shown in Figure 1.

IL-2 levels are depicted in Figure 2. In normal healthy patients, this cytokine is usually undetectable. The IL-2 results are similar to IFN-γ, in that a subset of both groups had detectable levels with SCD subjects showing 12 (21%) of 58 (range 2-27 pg/mL) and control subjects 18 (41%) of 43 (range 1-137 pg/mL).

Figure 3 depicts the results of sIL-2R. Analysis of the combined age group data revealed that the mean titer of the SCD group (4465±552 pg/mL) was significantly higher (P <.0001) than the normal group (3473±411 pg/mL). On closer analysis by age in the 1 to 17-year age group, there was no significant difference between the SCD subjects (4512±805 pg/mL) and the normal control subjects (4668±558). Conversely, in the older age group (18-45 years), the SCD individuals had much higher levels of sIL-2R than control subjects (4410±754 pg/mL versus 1789±345 pg/mL, respectively). No differences were seen that could be attributed to ethnicity in any of the comparisons.

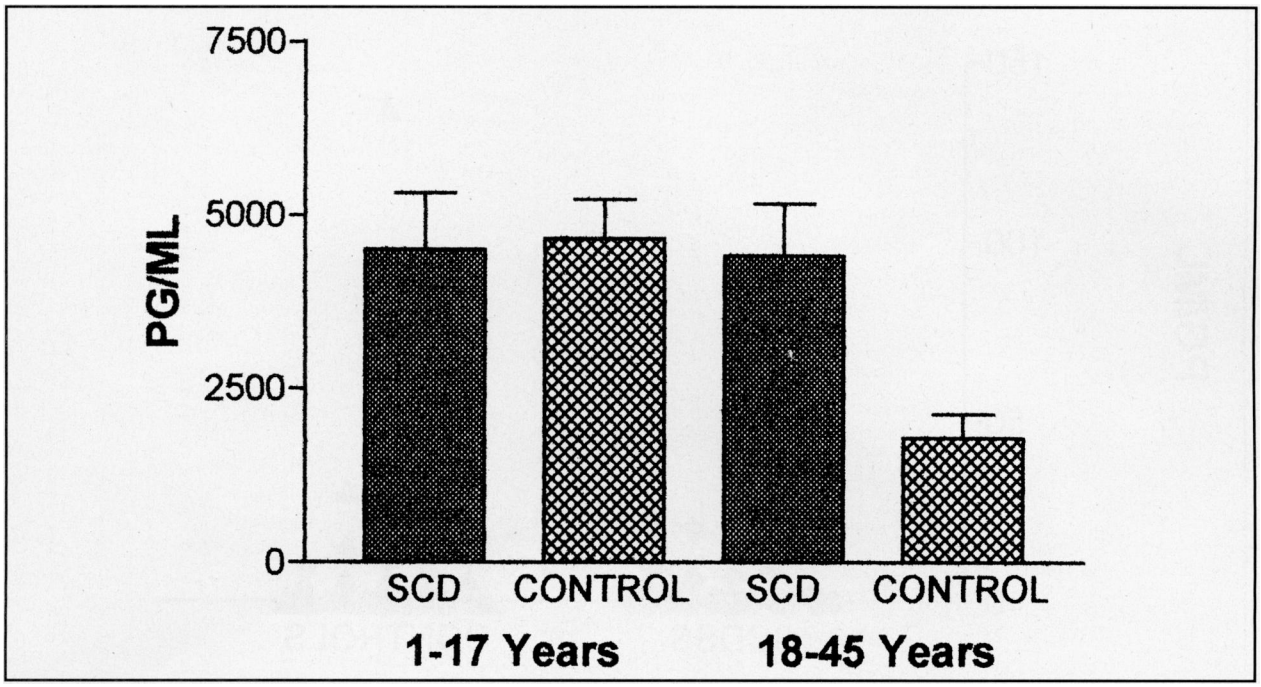

Figure 3. Individual soluble IL-2R serum level determinations were done by ELISA. The ranges of detectable individual levels included: 31 SCD steady-state patients age 1-17 years (381-18,416 pg/mL), 24 normal control subjects age 1-17 years (965-11,266 pg/mL), 26 SCD steady-state subjects age 18-45 years (673-13,991 pg/mL), and 18 control subjects 18-45 years (460-5434 pg/mL). The mean titer of the SCD group (4465±552 pg/mL) was significantly higher ($P<.0001$) than the control group (3473±411 pg/mL) combining all age groups by paired *t* test. The graph depicts the results by age grouping separately, demonstrating that the major difference between SCD (4410±754 pg/mL) and the controls (1789±345 pg/mL) is in the older age group.

DISCUSSION

The cytokines evaluated in this current study are important mediators of host inflammatory response. Recent paradigms characterize cytokines involved in type 1 and type 2 immune responses.[21-23] The type 1 pattern involves CMI, with IL-2 and IFN-γ being the principal autocrine growth factors to stimulate helper T cells in their activation of macrophages. Type 2 response cytokines, such as IL-4, IL-6, and IL-10, tend to communicate with suppressor T cells and drive the response toward humoral immunity. In addition, immune response depends on a balance of two T-helper cell subsets designated Th1 and Th2. Th1 cells synthesize and secrete the type 1 cytokines IL-2 and IFN-γ. Conversely, Th2 cells synthesize and secrete IL-4, IL-6, and IL-10. This rather simplistic model may have some validity but cannot completely explain the complexities of inflammatory response.

IL-1 and IL-12 play pivotal roles in type 1 immune responses. Both cytokines are mononuclear phagocytic cell-derived peptides. An important biological activity of IL-1 is its function as a lymphocyte activating factor. IL-1 enhances the production of IL-2 and the expression of IL-2R The biology of IL-12 relates to the regulation of IFN-γ production.[24-26] Like IL-2, IL-12 stimulates the differentiation of natural killer cells into lymphokine activated killer cells. Other functions attributed to IL-12 include stimulation of hematopoiesis and proliferation of CD4 and CD8 lymphocytes.

The lack of significantly increased in vivo production of the type 1 cytokines was somewhat surprising. The only exceptions were two SCD patients with high IFN-γ levels and the adult age sIL-2R levels in the SCD group. Previous data imply SCD even in the healthy state represents chronic inflammation.[14,16,20,27] The significance of the extremely high serum levels of IFN-γ seen in two healthy SCD individuals is not clear at this point. Serum levels of IFN-γ in this range may be an explanation for the impaired in vitro production of IFN-γ by a subset of healthy SCD patients previously

reported in the literature.[11,15] However, high circulating levels of type 2 cytokines may partially explain the present results showing high sIL-2R levels.[19] In a prior study of SCD patients, increased in vivo production of IL-6 was seen in 78% of patients and 41% had elevated levels of IL-10.[19] It is clear that IL-10 is a potent inhibitor of CMI and type 1 cytokines.[19] IL-6 mediates B-cell differentiation into plasma cells and antibody secretion favoring humoral immunity and can also increase IL-2 and sIL-2R expression. Increased circulating levels of TNF-α can have the same effect on sIL-2R expression.[23,27]

Cytokines and immune activation markers like sIL-2R are produced by cells that mediate host responses to infection or inflammatory stimuli.28-33 It has been well established that cytokine and immune activation marker leads correlate with severity of disease and/or clinical outcome in HIV infection and inflammatory disorders.33 Assessment of the sIL-2R results reveal that there is no significant difference between the two groups in the pediatric age group, but there is a tremendous difference with immunologic maturation in the adult group. That difference may be because chronic infections like parvovirus B19, polyclonal activation of B cells, or defective regulation of antibody production in patients with SCD. The type 2 cytokine predominance seen in a significant percentage of healthy SCD patients in combination with elevated sIL-2R levels may adversely affect CMI and humoral responses in SCD, with resultant increased morbidity.

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