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Increased serum soluble interleukin-2 receptors in schizophrenic monozygotic twins

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Summary. There is a confusing history of immune findings associated with schizophrenia. At least some of these discrepant results may be artifacts caused by heterogeneity. In an attempt to decrease heterogeneity, we studied three groups of monozygotic twins who were either discordant for schizophrenia, concordant and ill, or concordant and well. This comparison minimizes environmental and genetic variance, and heightens differences that are actually due to the disorder. Overall, schizophrenic subjects had higher levels of serum soluble interleukin-2 receptors (SIL-2Rs) than unaffected individuals (480.8, SD 238.6 U/ml vs 380.9, SD 170.6 U/ml; $F = 5.256$, $df = 1.61$, $P = 0.02$). When data from discordant and concordant twin groups were analysed separately, both the discordant ill twins ($P = 0.06$) and concordant ill twin pairs ($P = 0.08$) showed trends towards higher serum SIL-2R levels than their respective control groups. These data contribute to the growing body of evidence that immune activation is associated with some forms of schizophrenia.

Key words: Immunology – Schizophrenia – Twins – Soluble interleukin-2 receptors

Introduction

Schizophrenia is a heterogenous disorder which encompasses a variety of different symptom patterns, and, despite a 1% prevalence, its pathogenesis is not understood. One of the first biological findings ever reported in schizophrenia research was the presence of a leukocytosis in patients institutionalized because of acute psychosis (Bruce and Peebles 1903; Bruce and Peebles 1904; Dameschek, 1930; Dameschek 1931). Other investigations, before the introduction of neuroleptic medications, found both abnormally shaped peripheral lymphocytes and stem cells, and brain reactive auto-antibodies in the serum of schizophrenic subjects (Lehman-Facius, 1937; Lehman-Facius, 1939; Lepine and Popoff 1908; Lyubovskaya and Rokhlenko 1957). Unfortunately, follow-up studies could not replicate these findings. However, many of the investigations were confounded by methodological problems. Some of the more commonly observed problems included: 1. the effects of neuroleptics on immune function; 2. variability caused by differences in the diagnostic criteria used to define schizophrenia in these studies; 3. the racial and ethnic heterogeneity of subjects used in the studies; 4. the small number of subjects examined in many of the studies; 5. methodological and conceptional differences in the immune assays used in different studies (see Rapaport 1991b for review).

In the last decade, some investigators studying immune function in schizophrenia began taking into account these sources of experimental variability described above. They have consistently found signs of immune alterations consonant with immune activation in schizophrenic subjects. These include the presence of abnormal ratios of circulating lymphocyte subsets, increased peripheral auto-antibody production, and a decrease in the functional responsiveness of T-cells to mitogen challenge (Henneberg et al. 1990; Masserini et al. 1990; Spivak et al. 1991; McAllister et al. 1989b; Villemain et al. 1987; Ganguli et al. 1989b). In addition, our group and others have found that serum soluble interleukin-2 receptors (SIL-2Rs), a 45 kDa protein normally present at low levels in the peripheral blood, were significantly elevated in both Caucasian (Rapaport et al. 1989; Ganguli and Rabin 1989a) and non-

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Caucasian schizophrenic subjects (Rapaport, in preparation). SIL-2Rs have been extensively used in clinical and transplant immunology to monitor levels of immune activation (Rubin and Nelson 1990). They are elevated during the acute phase of some auto-immune disorders, during transplant rejection, infections, and in certain T-cell leukemias (Rubin and Nelson 1990; Manoussakis et al. 1989). Recent data have shown that SIL-2R levels in humans are not affected by the acute in vivo administration of neuroleptics (Rapaport et al. 1991a), and that neither duration of illness, nor gender correlate with serum SIL-2R levels (Rapaport, in preparation).

This study extends investigations of immune function in schizophrenia by contrasting serum SIL-2Rs in monozygotic twins. Three groups of twins were studied: twins discordant for schizophrenia, twins concordant for schizophrenia, and twins who were normal. (Monozygotic discordant twins are a particularly powerful population to study because they are genetically identical and have had similar in utero and postpartum experiences.) Previously published data from this cohort found that discordant ill twins had unusual magnetic resonance image scans, abnormal neuropsychological functioning, and an increased number of subtle neurological findings (Suddath et al. 1990; Casanova et al. 1990; Goldberg et al. 1990; Bracha et al. 1991). We hypothesized that monozygotic twins affected with schizophrenia would have increased serum SIL-2R levels as compared with the unaffected siblings, and normal twin pairs. We also postulated that monozygotic twins who are concordant for schizophrenia might have a different form of schizophrenia from monozygotic twins who are discordant for the disorder, and so we hypothesized that the two groups of twins might have different serum SIL-2R levels.

Methods

All subjects in each of the three twin groups signed informed written consent. One group consisted of 18 discordant monozygotic twin pairs: 10 female twin pairs and 8 male twin pairs. The second cohort consisted of ten pairs of twins concordant for schizophrenia and one concordant triplet. Nine of these twin pairs were male, one pair was female, and the triplets were female. The third group consisted of five pairs of concordant normal controls: four of these twin pairs were female and one pair was male.

Zygosity was determined by a physical likeness questionnaire, comparison of physical similarity on examination, and analysis of 19 red-cell markers. When necessary, both finger printing and HLA typing also were used to help determine zygosity (described in detail elsewhere). Subjects underwent a Structured Clinical Interview for DSM-III-R (SCID) to establish a DSM-III-R diagnosis (Spitzer et al. 1988).

Discordant monozygotic twins had a mean age of 30.00, SD 3.71 years. They had a mean duration of illness of 9.85, SD 3.88 years, and a mean neuroleptic exposure (in chlorpromazine equivalence) of 17,919, SD 27,894 mg. The mean age of ill concordant monozygotic twins was 30.65, SD 5.31 years. The mean duration of illness was 11.91, SD 7.00 years, and the mean neuroleptic exposure was 44,978, SD 55,107 mg. The mean age of the normal control twin set was 27.67, SD 2.58 years.

All serum samples were frozen at –70° C prior to being assayed for SIL-2R levels. The SIL-2R assay is a sandwich enzyme immunoassay. An anti-IL-2R monoclonal antibody is coated onto the polystyrene microtiter wells. Either standard or samples are pipetted (50 µl) in duplicate into the antibody coated wells. One hundred µl of HRP conjugated anti-IL-2R antibody is added to each well and the plate is gently agitated for 15 s. Then the plate is covered and incubated at room temperature for 3 h on a rotator set at 100 rpm. The sample is aspirated from the wells and the plate is washed three times with 350 µl of phosphate buffered saline (PBS wash buffer). Next, 100 µl of chromogen solution (O-phenylenediamine) solution is added to the wells and incubated at room temperature for 30 min. Finally, 50 µl of 2 N H_2SO_4 is added to each well and gently mixed (Kurman et al. 1992). The plate is read in an automated ELISA reader set at a 490 nm wavelength of light. The values reported are a mean of the two readings. One unit is equivalent to 3 pg/ml.

The data in this study were analyzed using a 3×2 ANOVA, Kendall and Spearman correlation coefficients.

Results

The data were analysed using an ANOVA with concordance, illness state, and gender as dependent variables. There were no significant 3-way or 2-way interactions, but there was a main effect for the presence of schizophrenia ($df = 1.61$, $F = 5.256$, $P = 0.02$). Schizophrenic subjects had a mean serum SIL-2R level of 480.0, 238.6 U/ml (mean, SD) vs 380.9, 170.6 U/ml for normal controls. When the 18 pairs of discordant monozygotic twins were analysed separately, there was a trend toward the affected twin having higher serum SIL-2R levels than the unaffected twin (579.8, 288.5 U/ml vs 433.7, 158 U/ml; $df = 1.34$, $F = 3.551$, $P = 0.06$). When concordant ill subjects were contrasted with concordant normal subjects, there was a trend toward affected individuals having higher SIL-2R levels (403.2, 157.7 U/ml vs 301.9, 163.6 U/ml; $df = 1.33$, $F = 3.187$, $P = 0.08$) (Fig. 1). Neither the duration of illness nor the previous history medication exposure correlated with serum SIL-2R levels (Kendall correlation coefficients were $r = -0.1808$, $P = 0.200$, and $r = 0.1304$, $P = 0.239$, respectively; Spearman correlation coefficients $r = -0.2586$, $P = 0.193$, and $r = 0.1805$, $P = 0.265$).

There was also a main effect for concordance ($df = 1.63$, $F = 9.795$, $P = 0.003$). Discordant ill twins had serum SIL-2R levels that were significantly higher than ill concordant twins (579.7, 288.5 U/ml vs 403.3, 157.7 U/ml, $df = 1.24$, $t = 2.34$, $P = 0.03$). Discordant unaffected twins had serum SIL-2R levels markedly higher than normal control twins (433.7, 157.9 U/ml vs 301.8, 163.6 U/ml, $df = 1.28$, $t = 2.21$, $P = 0.04$).

In an effort to see whether patients with clearly elevated serum SIL-2Rs differed clinically from those with "nor-

mal" SIL-2R levels, discordant schizophrenic twins with serum SIL-2R levels greater than one standard deviation above the mean value of the unaffected twin ($n = 6$) were contrasted with schizophrenic pairs where the ill twin had SIL-2R levels lower than or similar to the well twin ($n = 7$). Five pairs were omitted from this comparison because they fell in between the two groups. Although the sample size was too small for statistical analysis, the schizophrenic subjects with "elevated" serum SIL-2Rs had earlier ages of onset, higher negative symptom scores (SANS), more "soft" neurological signs, had spent more time in the hospital and had lower Axis V (functioning) scores. When the two groups were contrasted on possible etiological variables, those with "elevated" serum SIL-2Rs had fewer structural differences on MRI, fewer minor physical anomalies or obstetrical complications, and weighed slightly more at birth.

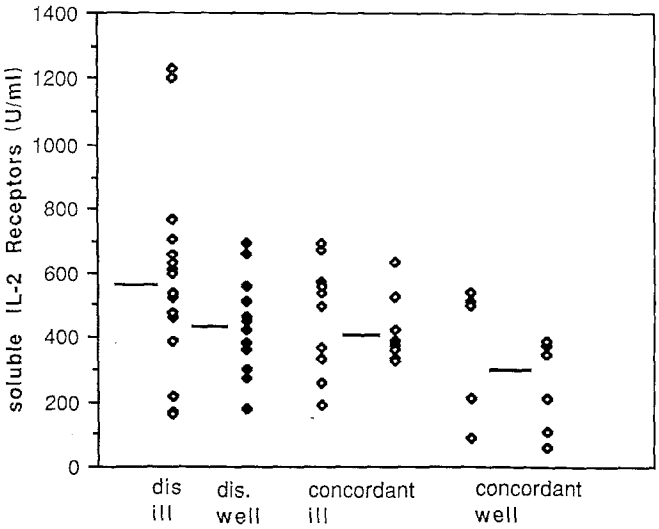

Fig. 1. This scattergram illustrates the differences in soluble interleukin-2 receptor levels in monozygotic twins discordant for schizophrenia and monozygotic twins concordant either for the presence or absence of schizophrenia. The *bars* represent the mean soluble interleukin-2 receptor values for the respective groups

Discussion

There have been many studies of immune abnormalities in schizophrenia; however, they have frequently reported discrepant findings. Some groups found evidence of immune activation in psychotic patients, while other groups did not (see Rapaport and McAllister 1991b; DeLisi 1986 for review). Heterogeneity may play a major role in causing these disparate results. We, and others, find that Caucasian schizophrenic patients have immune system abnormalities similar to those seen in autoimmune disorders namely increased numbers of CD5 positive B lymphocytes, and elevations in serum SIL-2Rs (McAllister et al. 1989b; Rapaport et al. 1989; Ganguli et al. 1989a). Studies have also demonstrated that neither of these immune abnormalities can be attributed to acute treatment with antipsychotic medication (McAllister et al. 1989a; Rapaport et al. 1991a). The current experiment extends investigations of the immune system in schizophrenia by studying monozygotic twins.

Serum SIL-2Rs were elevated in schizophrenic subjects as compared with control subjects (Fig. 1). Despite a small sample size, when data comparing SIL-2R levels in the monozygotic twins discordant for schizophrenia were analyzed, there was a trend toward schizophrenic subjects having higher serum SIL-2R levels ($P = 0.06$). A similar trend was seen when the twins concordant for schizophrenia were contrasted with the concordant well twin controls ($P = 0.08$). When viewed as a whole, these data are consistent with previously published material, and the results from a larger currently submitted study, which find significant elevations of serum SIL-2R levels in schizophrenic patients (Rapaport et al. 1989; Ganguli et al. 1989a, Rapaport, in preparation). Future studies will not only enable us to evaluate whether serum SIL-2R levels will be a useful technique for biologically subtyping schizophrenia, but will begin to investigate the source of this aberrant immune activation. It is possible that patients with increased serum SIL-2Rs may be the same patients that Villemain et al. (1987) and Ganguli et al. (1989b) found to have decreased T-lymphocyte mitogen-stimulated cytokine production. Such a linkage between our work and theirs would indicate that the lower levels of in vitro stimulated cytokine production seen are actually secondary to pre-existing in vivo immune activation, as is seen in active autoimmune conditions, rather than an intrinsic defect in IL-2 production (Rubin and Nelson 1990).

Two separate analyses were performed as part of an attempt to correlate clinical variables and serum SIL-2Rs. Neither the duration of illness nor the amount of prior medication exposure correlated with SIL-2R levels. In a second analysis, the discordant schizophrenic cohort was divided into "high" ($n = 6$) and "low" ($n = 7$) SIL-2R groups. Although these groups were too small to be statistically analyzed, subjects in the "high" SIL-2R group, generally, had an earlier age of illness onset, were sicker, more dysfunctional, had more negative symptoms, and more "soft" neurological signs. A larger sample where these factors are identified a priori needs to be studied before these potential clinical associations can be considered meaningful.

There was a significant main effect between concordance and serum SIL-2R levels: discordant twins had higher serum SIL-2R levels than concordant twins. Both the affected discordant twins and the normal discordant twins had higher levels of serum SIL-2Rs than affected concordant twins and normal control twins. These data could be an indication that more individuals in the monozygotic discordant group have a mildly activated immune system. This activation could be caused by intrinsic differences in immune system recognition factors such as HLA alleles or T-cell receptor subtypes. Thus, it is possible that the elevations in serum SIL-2R levels observed in some of the discordant ill monozygotic twins are due to an aberrant immune activation which may somehow be related to schizophrenia. At this time, it is not clear whether the presence of schizophrenia is somehow triggering immune activation, or if the immune activation is somehow involved in causing schizophrenia. Larger

studies specifically investigating this hypothesis are needed to validate this interpretation.

In conclusion, schizophrenic subjects had elevated serum SIL-2R levels. When twin pairs that are discordant for schizophrenia are evaluated, the ill twin manifests a trend toward higher serum SIL-2R levels than the well twin. When ill and well concordant monozygotic twins are contrasted, affected subjects also have a trend toward higher serum SIL-2R levels than normals. The analyses of clinical data did not find significant correlations with SIL-2Rs; however, there are indications that certain clinical factors might be used to describe discordant monozygotic twins with higher SIL-2R levels. Further studies are needed to clarify the significance of increased SIL-2Rs in schizophrenia.

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