Leukocytes and Organ-Nonspecific Autoantibodies in Schizophrenics and Their Siblings: Markers of Vulnerability or Disease?

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To determine whether leukocyte counts and organ-nonspecific autoantibodies mark familial vulnerability for schizophrenia and/or the disease itself, we examined 92 patients with schizophrenia and 94 unrelated, demographically balanced, healthy individuals. In addition, for 19 of the probands, one of their nonschizophrenic, full siblings also was recruited. At the time of the blood draw, most probands (87%) had been free of medications for a minimum of 2 weeks and about half were neuroleptic-naive, first-episode patients. Results indicate that a relative lymphopenia in the context of a relative granulocytosis appears to mark familial vulnerability for schizophrenia, whereas an absolute monocytosis appears to mark spectrum manifestations of the clinical phenotype. The former observation is consistent with the hypothesis that the etiology of schizophrenia is immunologically mediated, whereas the latter is consistent with emerging evidence that an inflammatory process is associated with the expression of the disorder. Neither antinuclear antibody nor rheumatoid factor emerged as liability or disease markers. © 1996 Society of Biological Psychiatry

Key Words: Leukocytes, lymphocytes, granulocytes, monocytes, autoantibodies, family study

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Introduction

Hundreds of studies have documented immunological abnormalities in patients with schizophrenia, though their causal significance remains poorly understood. One approach toward understanding their etiologic significance is to determine their presence in well relatives of schizophrenic patients. Because schizophrenia is a partly heritable disorder in which environmental factors moderate disease expression, some unaffected first-degree relatives would be expected to manifest traits associated with genetic liability, even though they do not show the clinical syndrome (Cannon and Marco 1994). Thus, if immunological sensitivity represents a vulnerability for schizophrenia, as it does for many recognized autoimmune disorders amenable to similar diathesis/stress models (Bach 1994; Harley et al 1994; Moreland and Koopman 1991; Panitch 1994), one should observe immunologic liability markers in some unaffected family members. Such abnormalities could play a role in the pathophysiology of the disorder, or merely could mark vulnerability for

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schizophrenia (Cannon et al 1994; DeLisi et al 1987; Spring and Zubin 1978; Tsuang et al 1990). On the other hand, if an abnormality is specific to the schizophrenic phenotype and is not evident in well family members, it is likely to be relatively remote from genetic risk factors for the disease. Such disease markers, however, might play an important part in the disorder's pathophysiology.

Discordant siblings provide an opportunity to search for endophenotypic (liability) and phenotypic (disease) markers (Cannon et al 1994). Well siblings are more likely to exhibit liability markers than unrelated, healthy individuals. Concomitantly, studies of discordant siblings are more likely to detect disease markers than those that utilize unrelated controls; this greater sensitivity is achieved because full siblings control for a significant proportion of random familial variability. Indeed, previous studies have shown that the sensitivity of putative disease markers of schizophrenia is increased when *pairwise* analyses are performed on related siblings, promoting the value of controlling for familial variability (Cannon et al 1994; Eyler Zorrilla and Cannon 1995). Herein, we extend this logic to candidate immunologic markers of vulnerability and disease.

Supporting the possibility that immune disturbance is related to liability for schizophrenia, elevations in serum levels of soluble interleukin-2 receptor (sIL-2r), titers for selected natural autoantibodies, and numbers of circulating atypical lymphocytes have been reported in nonschizophrenic relatives of schizophrenic probands (Hirata-Hibi and Hayashi 1993; Rapaport et al 1993; Sirota et al 1993a, 1993b); however, conclusions from these findings are limited by the studies' methodologies. These include a failure to determine the presence of spectrum disorders; the use of between-family, rather than pairwise, familial analyses; and the use of multiplex families (Sirota et al 1993a, 1993b) and twin births (Rapaport et al 1993). Collectively, these concerns question the generalizability and the classification of these putative risk markers. To address these limitations, we have examined selected immunological markers in discordant siblings from families unselected for familial risk; further, we have considered the relation of spectrum psychopathology to immunological deviation in the nonschizophrenic siblings.

Though they have been used routinely to screen for medical conditions that could complicate treatment or research, relatively little is known about absolute and differential white blood cell (WBC) counts in schizophrenia. In contrast, many studies of leukocyte alterations in stressed and depressed individuals have been conducted. Recently, we meta-analytically reviewed these literatures and confirmed that, relative to one another, stressors and major depression are associated with nonidentical aberrations in WBC counts (E.P. Zorrilla unpublished observations). As such, unique aberrations in WBC counts could be associated with psychotic symptomology as well. Early reports of leukocyte counts and differentials in schizophrenia did not observe consistent differences—one noted an absolute neutropenia and lymphocytosis in a subset of patients (Dameshek 1930), whereas another found no relation between WBC counts and psychiatric status (Milhorat et al 1942). Contemporary studies, which rely on more reliable diagnostic criteria, have not revealed a more consistent picture. Kronfol and colleagues (Kronfol et al 1984, 1986) reported an absolute leukocytosis in 178 untreated schizophrenics relative to normative ranges (Osgood et al 1939). Differentials indicated an absolute and proportional neutrophilia coupled with an absolute and relative lymphopenia; however, no normal control values were provided from the authors' own laboratory, thereby questioning the validity of the findings. These concerns are amplified in that others have not observed a leukocytosis, neutrophilic or otherwise (Caldwell et al 1991; Diebold 1976; Sasaki et al 1994). In addition, *increased* (Masserini et al 1990) and normal lymphocyte counts (Achiron et al 1994; Coffey et al 1983; DeLisi et al 1982; Diebold 1976; Nyland et al 1980; Sasaki et al 1994) have been reported; however, consistent with Kronfol's findings, other (Sasaki et al 1994; Winokur and Tsuang 1981), but not all (Caldwell et al 1991), studies have reported values that indicate a relative lymphopenia, though this difference may be specific to nonparanoid patients (Winokur and Tsuang 1981). Possible explanations for these discrepant findings include differences in sample size; adequacy of control matching; medication history, chronicity, and clinical symptomology of patients; and reliability of WBC counts (i.e., manual vs. electronic determination).

Organ-nonspecific autoantibodies, in particular antinuclear antibody (ANA) and rheumatoid factor (RF), have received attention as putative evidence of autoimmune-like activity in schizophrenia. Many studies have documented an increased prevalence of ANA positivity in schizophrenic patients (Canoso and de Oliveira 1986; Canoso et al 1990; Canoso and Sise 1982; DeLisi and Wyatt 1982; Fessel 1961; Ganguli et al 1992b, 1993; Gottfries and Gottfries 1974; Johnstone and Whaley 1975; Sirota et al 1993b; Yannitsi et al 1990; Zarrabi et al 1979); however, several others have found no relation (Chengappa et al 1991; Villemain et al 1988, 1989; von Brauchitsch 1972), including the largest existing study of never-medicated patients (Ganguli et al 1993). In addition, only two studies (DeLisi and Wyatt 1982; Sirota et al 1993b) tend to refute the argument that elevated ANA titers are medication artifacts, which are not related to the disease's etiology or pathophysiology. Similar criticisms confront studies that have found an increased frequency of

Table 1. Demographic Characteristics of Schizophrenic Probands, Siblings, and Normal Controls

Characteristic	Probands			Siblings	Controls
	All (n = 92)	With sibling (n = 19)	Without sibling (n = 73)	(n = 19)	(n = 94)
Sex (%)					
Female	41	37	42	42	45
Male	59	63	58	58	55
Race (%)					
African-American	53	53	53	53	46
White	47	47	47	47	54
Handedness (%)					
Right	83	74	84	86	89
Nonright	17	26	16	14	11
Age (years)	30.5 ± 9.0	30.7 ± 8.7	30.4 ± 8.4	31.1 ± 10.3	29.1 ± 6.9
Parental education (years)	12.3 ± 2.6	12.9 ± 2.5	12.3 ± 2.6	12.9 ± 2.5	11.9 ± 2.0

Note: Continuous variables presented as mean ± SD.

positivity for RF titers (Canoso et al 1990; Zarrabi et al 1979). Many studies have failed to find such a relation, including the largest study of neuroleptic-naive patients (Chengappa et al 1991; Ganguli et al 1992b, 1993; Yannitsi et al 1990), and no study counters the argument that positive findings represent a medication artifact.

To address the concerns outlined above, we have determined WBC counts and positivity for ANA and RF in a large sample of predominantly drug-free schizophrenic patients and compared them to those from a subset of their nonschizophrenic siblings as well as to a large group of demographically balanced healthy controls. Based on the extant literature we made the following predictions: (1) neutrophilic leukocytosis and a concomitant relative lymphopenia are phenotypic (disease) markers of psychotic symptomology; therefore, patients will exhibit this leukocyte profile when compared to unaffected siblings and to demographically matched normal controls, who will not differ from one another; and (2) ANA positivity is an endophenotypic (liability) marker, whereas RF positivity is a consequence of chronic neuroleptic treatment; therefore, patients and their siblings will obtain a similarly elevated frequency of positive ANA titers relative to normal controls, whereas RF titers will be similarly negative across all groups.

Methods

Subjects

The primary sample consisted of 92 schizophrenic probands and 94 normal controls unrelated to the patients. For 19 of the probands, one of their nonschizophrenic full siblings was recruited for the study. All subjects were recruited and evaluated by the Mental Health Clinical Research Center (MHCRC) in the Department of Psychiatry at the University of Pennsylvania. Participants underwent a psychiatric interview (Structured Clinical Interview for DSM-III-R, Patient or Nonpatient Edition; Spitzer et al 1987a) and physical examination, including routine laboratory tests (Shtasel et al 1991). Exclusion criteria were major medical illnesses, including those recognized to alter immunologic functioning, current or past substance abuse disorder, neurologic condition, including history of head injury with loss of consciousness, and age of less than 17 or greater than 55 years (Cannon et al 1994). Probands met DSM-III-R criteria for schizophrenia (n = 88) or schizoaffective disorder (n = 4), with no concurrent diagnoses. Siblings were within 5 years of age of their proband and free of any axis I diagnosis. Normal controls were free of any axis I disorder as well as a family history of schizophrenia or affective illness.

Normal controls were matched to probands on the following variables: age (within 5 years), sex, race, handedness, and parental educational level (within 2 years of the parental average). Good matching was achieved across the groups (see Table 1), and no differences approached significance (all $ps > .10$). Furthermore, probands with siblings in the study did not differ significantly on the matching variables from probands who did not have siblings in the study (all $ps > .10$). Finally, no significant differences existed between probands, controls, or siblings with respect to season or cross-season of assessment (all $ps > .4$; Maes et al 1994b).

The 92 probands in our study include all eligible, willing MHCRC probands, mostly recruited over a 5-year period extending through August, 1994. Their mean (± SD) age of onset was 23.4 ± 7.2 years, and their mean duration of illness was 6.5 ± 7.1 years. At the time of the blood draw, most probands (87%) had been drug free for at least 2 weeks, and 42 (46%) were first-episode, neuro-

leptic-naive patients. Analyses that utilize only never-medicated subjects show equivalent effect sizes to those performed with the entire patient sample; furthermore, they do not differ significantly from those that include only previously medicated subjects. Therefore, we report results for the entire sample.

Beginning in 1993, available, eligible full siblings of admitted probands were asked to participate. Full siblings closest in age to and of the same sex as their affected family member were recruited preferentially. Most non-schizophrenic siblings (79%) were the same sex as their affected family member, and good age-matching was achieved as well (see Table 1). Each sibling underwent assessment of axis II psychopathology by structured interview (Loranger et al 1987; Spitzer et al 1987b). Four siblings met criteria for a probable or definite diagnosis of schizotypal personality disorder.

Procedures

All hematologic and serologic analyses were performed in the Clinical Immunopathology Laboratory of the Hospital of the University of Pennsylvania. Total and differential WBC counts were determined with an impedance cell counter (Coulter Counter Model S-Plus KS, Coulter Electronics, Florida). The interassay coefficient of variation for total leukocyte counts for this counter is 1.3%; standard deviations for 31 retest replicates for lymphocytes, monocytes, and neutrophils are 0.55%, 0.33%, and 0.69%, respectively. ANA and RF titers were semiquantitatively determined using commercially available kits following recommended procedures (Antibodies Incorporated, Irvine, CA and Behring Diagnostics, Somerville, NJ, respectively). Observed results were compared to normative laboratory standards to determine positivity; the clinical laboratory regularly exhibits satisfactory accuracy and reproducibility in external blind proficiency tests for these autoantibody assays. Only a subset of subjects have data for autoantibody titers, as they were not quantitated at the outset of this study. Reduced degrees of freedom in the hematologic analyses similarly reflect the fact that total, but not differential, leukocyte counts were performed on the earliest recruited subjects.

Statistical Analyses

Proband–control hematologic differences were evaluated through application of the general linear model. To reduce the likelihood of making a type I error, multivariate analyses of covariance (MANCOVAs) were performed to test for group differences on the absolute and differential counts, with age, sex, and race as covariates. Follow-up univariate analyses (two-factor ANCOVAs with age and race as covariates and sex as the second factor) were performed only when the omnibus test was significant. To assess the possible role of medication effects in observed differences, we compared neuroleptic-naive patients to previously medicated patients as well as to normal controls. To assess the potential classification utility of the absolute leukocyte measures (i.e., total granulocytes, lymphocytes, and monocytes), we performed a canonical (parametric) linear discriminant analysis (LDA), using the residuals of these measures after controlling for differences in age, sex, and race. Candidate markers were classified tentatively as liability and/or disease markers using proband–sibling paired t tests and sibling–control two-factor ANCOVAs (covarying for age and race with sex as second factor). Finally, we compared characteristics of incorrectly classified probands to those of correctly classified probands through t tests, to determine whether the markers might be more useful (or relevant) for particular populations.

To characterize further the validity of the candidate liability markers, we explored their relation to schizotypal personality disorder (SPD) using t tests (Kruskal–Wallis tests were used when distributional requirements for parametric testing were not met). First, we determined whether the putative liability markers only appeared aberrant in siblings with SPD as opposed to non-SPD siblings—a finding that could suggest they reflected manifest, rather than latent, psychopathology. Second, we determined whether these markers were more aberrant in probands who had siblings with SPD than in those who did not—a finding that could provide converging evidence for their role as markers of familial risk for schizophrenia spectrum disorders. Parallel analyses were performed for the disease markers to determine if siblings with manifest psychopathology differed from controls and unaffected siblings, as would be expected from a phenotype marker. These analyses were performed on a small sample of siblings and should, therefore, be considered exploratory and preliminary.

We used the χ^2 statistic or Fisher's Exact Test for comparisons with small ($n < 5$) expected cell size to examine groupwise differences in rates of autoantibody positivity (i.e., ANA+, RF+, or positive for either autoantibody). Because the clinical laboratory's conservative ANA+ threshold (1:160) tended to be overinclusive, duplicate analyses were conducted using a 1:320 dilution as a positive threshold, which yielded more typical rates of ANA+ controls. Because of the small number of ANA+ patients obtained using the stricter criterion, we did not explore alterations in leukocyte subsets according to ANA. The statistical package used was SAS 6.09 (SAS Institute 1988).

Table 2. Total, Absolute, and Differential WBC Counts in Schizophrenics, Their Siblings, and Normal Controls

WBC	Probands	Controls	Siblings	Probands − siblings[a]	Marker?
Leukocytes $10^3/\mu l$[b]	7.4 ± 2.3	6.3 ± 2.0	6.8 ± 2.2	1.3 ± 3.2	Disease?
Granulocytes					
Total μl[c]	4906 ± 2177	4066 ± 1726	4400 ± 1521	959 ± 3342	Both?
%[d]	64.6 ± 10.2	61.3 ± 10.1	64.4 ± 7.4	0.3 ± 3.5	Liability?
Lymphocytes					
Total μl	2010 ± 571	2053 ± 569	2011 ± 777	5 ± 799	No
%[e]	29.1 ± 9.8	33.8 ± 8.6	29.6 ± 6.0	−2.5 ± 12.6	Liability
Monocytes					
Total μl[f]	383 ± 193	308 ± 156	337 ± 171	117 ± 154	Disease
%	5.6 ± 2.7	5.2 ± 2.7	5.3 ± 2.4	1.2 ± 3.6	No

Note: All values are expressed as mean ± SD.
[a]Difference between probands and their paired nonschizophrenic sibling.
[b]Probands > siblings = controls ($p < .10$ and $p < .0025$ relative to siblings and controls, respectively).
[c]Probands > controls ($p < .01$), with siblings nonsignificantly intermediate.
[d]Probands > controls ($p < .05$), with siblings nonsignificantly more like probands than controls.
[e]Probands = siblings < controls ($p = .001$ and $p < .05$ relative to probands and siblings, respectively).
[f]Probands > siblings = controls ($p < .01$ and $p < .005$ relative to siblings and controls, respectively).

Results

WBC Counts

CANDIDATE MARKERS IN PROBANDS. Probands had more total leukocytes than controls [$F(1,177) = 9.67, p < .005$]. Furthermore, their absolute [MANCOVA: $F(3,147) = 5.17, p < .005$] and differential [MANCOVA:$F(3,147) = 4.47, p < .005$] leukocyte subset counts also differed from those of controls. As depicted in Table 2, probands exhibited an absolute excess of both granulocytes and monocytes, with no concomitant change in circulating lymphocyte numbers. Consequently, they exhibited a relative granulocyte excess and a relative lymphocyte deficit. Follow-up analyses based on laboratory cutoffs for normal ranges confirmed that probands were significantly more likely to exhibit a relative granulocyte excess (18% vs. 6%, $p < .05$) and lymphopenia (21% vs. 4%, $p < .005$) than normal controls. None of these differences appeared to be artifacts of medication treatment, as neuroleptic-naive patients did not differ from previously medicated patients (all ps $> .10$), and both groups showed similar effect sizes when compared to controls. Furthermore, these differences were not specific to the patient's sex (all Sex × Diagnosis ps $> .10$).

A canonical linear discriminant analysis (LDA) revealed that absolute leukocyte subset counts could be used to identify the majority of probands with some specificity (sensitivity = 62%, specificity = 75%, PV+ = 75%; distance between centroids = 0.75 SDs; canonical $r = 0.35$). As suggested by the univariate analyses, the LDA differentiated subjects according to granulocyte ($r = 0.70$) and monocyte ($r = 0.74$), but not lymphocyte ($r = -0.04$), counts. Those probands missed by the classification function did not differ significantly from correctly detected probands in any matching variables, nor in their frequency of autoantibody positivity (all ps $> .10$).

CANDIDATE MARKERS IN SIBLINGS. Like probands, siblings exhibited a significant relative lymphocyte deficit compared to controls (see Table 2). Though they showed elevated relative and absolute numbers of granulocytes, these differences did not reach significance. Probands tended to have more circulating leukocytes than their matched siblings, as was observed when they were compared to normal controls. Also as observed relative to controls, this difference was significant for the monocyte subset (see Table 2).

Thus, a relative lymphocyte deficit, in the context of a relative granulocytosis, emerged as the strongest candidate indicator of liability, since both probands and nonschizophrenic siblings differed significantly from controls on this marker. In contrast, total monocyte and, possibly, leukocyte counts, on which probands differed from both normal controls and their siblings, emerged as the strongest candidate disease markers. Finally, total granulocyte counts emerged as a possible indicator sensitive to both liability and disease (see Table 2).

RELATION OF SPECTRUM PSYCHOPATHOLOGY TO THE LIABILITY MARKERS. The putative liability markers did not appear to be artifacts of subclinical psychopathology in the siblings. Most critically, non-SPD siblings ($n = 15$) showed proportional granulocyte (66.1 ± 6.9) and lymphocyte (28.0 ± 5.2) values that were similar to those of their affected sibling (ps $> .5$) and that deviated from those observed in normal controls [$F(1,87) = 3.10, p = .08$ and $F(1,87) = 6.21, p < .05$, respectively]. Also, probands who had siblings with SPD, a putative indicator of increased familial risk, exhibited proportionally more granulocytes and fewer lymphocytes than probands whose siblings did not have SPD, further strengthening the possibility that they marked familial risk [Kruskal–Wallis $\chi^2(1)$s $> 3.70, p$s $\leq .05$]. Finally, total granulocyte counts

Table 3. Prevalence of Organ-Nonspecific Autoantibodies in Schizophrenics, Their Siblings, and Normal Controls

Autoantibody	Probands	Siblings	Controls
ANA			
1:160 cutoff	7/56 (13%)	5/19 (26%)	18/84 (21%)
1:320 cutoff	1/56 (2%)	3/19 (16%)	5/84 (6%)
RF	3/33 (9%)	3/18 (17%)	5/74 (7%)
ANA or RF[a]			
1:160 cutoff	9/38 (24%)	7/18 (39%)	21/74 (28%)
1:320 cutoff	4/34 (12%)	5/18 (28%)	9/73 (12%)

Note: All results are expressed as no. positive/no. tested (% positive).
[a]Includes subjects who were tested for only one autoantibody and obtained a positive result as well as subjects who were tested for both. Cutoffs reflect ANA dilutions.

did not appear to be a sensitive liability marker, as non-SPD siblings exhibited levels (4373 ± 1491) that were equivalent to control levels (Effect Size < 0.3 SDs, $p > .5$).

RELATION OF SPECTRUM PSYCHOPATHOLOGY TO THE DISEASE MARKERS. Analyses that considered the presence of subclinical psychopathology further supported the validity of total monocyte counts as a disease marker. Non-SPD siblings exhibited control-like monocyte levels (313 ± 114, $p > .9$ relative to controls), which were on average more than one standard deviation below those of their schizophrenic sibling. In contrast, siblings with SPD exhibited probandlike monocyte levels (425 ± 189), that were reduced relative to those of their affected sibling by less than one third of a standard deviation. Absolute leukocyte and granulocyte counts did not appear to be sensitive disease markers, as probands of non-SPD siblings did not show levels that were appreciably greater than those of their unaffected sibling (mean pairwise differences < 0.3 SDs, $ps > .5$).

Organ Nonspecific Autoantibodies

CANDIDATE MARKERS. Groupwise, probands, siblings, and controls showed equivalent rates of positivity for the organ nonspecific autoantibodies (see Table 3; all group comparison $ps > .10$). Though probands and siblings had an elevated rate of ANA positivity relative to established population norms, this appeared to reflect an overly conservative threshold, as controls evidenced comparably high prevalence rates. Analyses that utilized the stricter cutoff dilution (1:320) obtained similarly negative results.

Discussion

The primary results of this study are that: (1) a relative lymphopenia in the context of a relative granulocytosis appears to mark vulnerability for schizophrenia; (2) an absolute monocytosis, associated with a further granulocytosis, marks manifest schizophrenic symptomology; and (3) absolute and differential WBC counts can discriminate the majority of schizophrenic patients from healthy controls with good specificity. In addition, consistent with the largest existing study of never-medicated patients (Ganguli et al 1993), we did not find an altered prevalence of ANA or RF in the probands or in their nonschizophrenic siblings, suggesting that neither autoantibody is generally associated with risk for the disorder or with the disease itself.

To be most useful, liability markers should be specific, sensitive, directly associated with degree of risk, and easily measured. Once identified, these markers can be used in etiologically oriented research that ultimately could yield improved treatments and preventative measures for the disorder. Proportional lymphopenia, in the context of a relative granulocytosis, preliminarily meets the last three criteria, but its specificity is uncertain. A meta-analytic review indicates that depressed patients exhibit identical alterations (E.P. Zorrilla unpublished observations), and many medical conditions are associated with perturbations of these cell populations as well. Hence, these markers are aspecific to schizophrenia and should not be used in isolation. Future research should determine their presence in well relatives of depressives to assess the possibility that they nonspecifically mark vulnerability for psychopathology.

Whether monocytosis is specific to schizophrenia or a generalized correlate of psychopathology is uncertain. Some have observed an absolute monocytosis in melancholia (Maes et al 1992), whereas others have observed reduced monocyte counts in depression (Kronfol and House 1989). Existing studies of bipolar patients (Diebold 1976; Kronfol et al 1986) have revealed decreased monocyte counts, suggesting that monocytosis is not entirely aspecific.

Consistent with previous research (Hirata-Hibi and Hayashi 1993; Rapaport et al 1993; Sirota et al 1993a, 1993b), we observed hematologic deviations in well relatives of schizophrenic patients, supporting the possibility that schizophrenia's etiology is immunologically mediated. Further, the present liability markers are not artifacts peculiar to twins or multicase families, nor are they the consequence of subclinical symptomology; however, given the bidirectional nature of neural–immune relations and the observation of increased ventricular volume in well relatives of schizophrenics (Reveley et al 1982; Weinberger et al 1981), these liability markers may reflect disturbed neural regulation of the immune system rather than (the potential for) pathological immune influences on the brain.

Our findings concur with the possibility that schizo-

phrenia is associated with an inflammatory response. First, an absolute expansion of the monocyte subset emerged as the most useful disease marker. Second, patients also evidenced an absolute granulocytosis. These increases in circulating phagocytic cells occurred in the absence of alterations in peripheral lymphocyte numbers. Though no existing studies of schizophrenia, to our knowledge, have observed a monocytosis, ours is the first large study of predominantly unmedicated patients to determine WBCs electronically and to utilize familial controls. Medication effects, unreliability of manual quantitation, or between-family variability may have obscured the monocytosis in previous studies. Increased phagocytic cell counts are consistent with reports of increased in vivo interleukin-6 (IL-6) levels (Ganguli et al 1994; Maes et al 1994a; Shintani et al 1991; but see Katila et al 1994; Xu et al 1994), as well as increased spontaneous ex vivo production of IL-1 and IL-3-like activity (Sirota et al 1995), in schizophrenic patients. Administration of IL-6 at pathophysiologically relevant doses induces an absolute neutrophilia (Asano et al 1990; Ulich et al 1989b, 1991) and monocytosis (Asano et al 1990). Furthermore, IL-3 promotes the proliferation and differentiation of the granulocytic cell line (Fishman et al 1990; Ulich et al 1989a), and its effects are augmented by IL-6 (Ulich et al 1989a). Therefore, future work should examine the relation of these, and other, interleukins, as well as their soluble receptors, to increased phagocyte counts. Such investigations should be extended to family studies, as the siblings' differentials revealed mild elevations in phagocyte counts, suggesting a continuum of inflammation-like activity. Furthermore, future longitudinal studies should investigate the effects of aging on the increased phagocyte numbers—decreased sIL-6r levels have been observed in older, but not younger patients (Maes et al 1994a). This suggests a blunting of immune activation in aging patients, as the IL-6/sIL-6r complex may promote signal transduction via the gp130 protein.

Unlike previous work (Sirota et al 1993b), we did not observe that ANA marked familial risk for schizophrenia. On the contrary, consistent with previous studies of unmedicated patients (Ganguli et al 1993), we observed normal prevalences of ANA and RF in both siblings and patients; furthermore, preliminary analyses do not indicate a familial concordance for ANA status (E.P. Zorrilla, unpublished observations). Possibly, the importance of ANA titers as a liability marker is circumscribed to multicase families or to a subgroup of patients who exhibit pathologic alterations in aspects of cellular immunity (Ganguli et al 1992a; Ganguli and Rabin 1993).

The possible effects of cigarette smoking should be considered when interpreting the present results. Smoking is more prevalent among schizophrenics, especially chronic, institutionalized patients (de Leon et al 1995), than the general population (Lohr and Flynn 1992). Tobacco use is a significant predictor of immune differences, including neutrophilia (Herbert and Cohen 1993), in major depression and stress (Irwin et al 1989); however, cigarette smoking also is related to altered lymphocyte counts (Herbert and Cohen 1993), which were unchanged in the present study, and the present sample was neither chronic nor institutionalized. Future studies should determine the contribution of chronic and acute effects of smoking to the present hematologic alterations as well as to previously reported immunologic differences in schizophrenia.

Using a discriminant function that relied on granulocyte and monocyte counts, we were able to identify the majority, but far from all, patients with some specificity. Physicians and researchers should be aware that these "aberrant" leukocyte profiles are normal for patients with schizophrenia and do not necessarily signal the presence of other medical conditions. On the contrary, leukocyte subset counts, combined with other, discriminating actuarial data, could be used to assist the diagnosis of schizophrenia.

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