Studies of Platelet Monoamine Oxidase Activity in Epstein-Barr and Dengue Virus Infections

by Edward G. Shaskan, Michael A. Peszke, and James C. Niederman

Abstract

As part of a prospective, psychosocial, and biochemical study of infectious mononucleosis, platelet monoamine oxidase (MAO) activity has been evaluated as a host factor. It was found that platelet MAO activity may be a possible predisposing host factor but not a precipitating factor. The results on infectious mononucleosis, a viral disease which involves the host's cell-mediated immune system, are compared with an evaluation of platelet MAO activity in dengue, a viral disorder involving the host's humoral immune system. The platelet MAO activity in these disorders has been compared to that in schizophrenia, a disease for which low platelet MAO activity has been postulated, from retrospective and twin studies, to be a risk factor. One hypothesis suggests that low platelet MAO activity predisposes to development of schizophrenia, but also increases cell-mediated immune system responses.

Stimulated by reports that platelet monoamine oxidase (MAO) activity was lower in chronic schizophrenics compared to controls (Murphy and Wyatt 1972) and that low platelet MAO activity may represent a genetic marker in the pathogenesis of schizophrenia (Wyatt et al. 1973), Shaskan and Becker (1975b) observed that platelet MAO activity in many controls was as low or lower than in schizophrenics. A study of 55 schizophrenic patients suggested that low platelet MAO activity may be a factor in the pathogenesis of schizophrenia (Becker and Shaskan 1977).

The rationale for presenting data on platelet MAO activity as a possible host factor in infectious disease at a symposium on schizophrenia may be related to issues of study design. The process and outcome may, at least, be of heuristic value.

Studies of chronic disease have been mainly retrospective and are problematic because the disease itself or its therapeutic management may alter host factors, but also the design of the studies may increase the probability of investigator bias. Bias may be expressed, for example, in the selection of cases (e.g., in case-control studies) for which there may exist a wide range of severity representing a spectrum of different diseases with different etiologies. Additionally, investigator bias may be expressed by the exclusion of certain cases which do not "fit the hypothesis."

More relevant to what we have learned from infectious disease epidemiology is that retrospective studies exclude all infections not manifested in disease—i.e., subclinical or inapparent cases. Retrospective studies exclude the possibility of delineating predisposing host factors.

If we postulate that low platelet MAO activity, in addition to psychosocial host factors, is predisposing to schizophrenia, then do "subclinical" cases of schizophrenia exist? Similarly, what are the precipitating host factors? Are subclinical cases of schizophrenia more prone to this or related psychiatric disease at some future time, i.e., in early adulthood or later during periods of high stress? Some of these questions might begin to be answered, if schizophrenia research were to borrow from the principles of epidemiology.

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Our original reasons for evaluating platelet MAO in infectious mononucleosis were related to clinical depression as a possible outcome in this disease. Despite the fact that our study population did not afford a reliable assessment of this outcome measure, platelet MAO activity initially appeared to distinguish between clinical and subclinical cases of this infectious disease (Shaskan et al. 1978). Therefore, stimulated by reports of modulation of host’s immune responses in mice chronically treated with L-dopa (Cotzias et al. 1977), we continued to study platelet MAO activity in infectious disease, extending observations to dengue fever.

Infectious mononucleosis (IM) is a disease primarily affecting young people in the age group of 15–25 years. The Epstein-Barr virus (EBV) has been shown to be the cause of heterophile-positive IM, and development of a highly specific fluorescent antibody technique for the presence of serum viral capsid antibody (VCA) as a marker of this infection has been useful in epidemiologic studies of clinically apparent and inapparent EBV infection. This study was started at the United States Coast Guard Academy (USCGA) in 1976, and data are being collected on three successive entering cadet classes.

Methods

Sera collected from cadets on entrance to the Academy are tested for the presence or absence of EBV-VCA antibody, and the number of susceptibles recorded. Yearly paired samples showing seroconversion establish the EBV infection rate in this population. Blood is also collected during interim illnesses and tested for EBV-VCA antibody, in order to diagnose the spectrum of clinical illnesses associated with EBV infection.

Entering freshmen cadets in three successive classes at the Academy in New London, Connecticut, were asked to participate in a psychobiological study of infectious mononucleosis. Blood was obtained from participating cadets for determinations of EBV-VCA antibody (Henle and Henle 1966) and

Table 1. Prevalence of the Epstein-Barr virus (EBV) viral capsid antibody

<table>
<thead>
<tr>
<th>EBV antibody status</th>
<th>Number</th>
<th>Percent</th>
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<tbody>
<tr>
<td>Negative</td>
<td>268</td>
<td>38</td>
</tr>
<tr>
<td>Positive</td>
<td>438</td>
<td>62</td>
</tr>
</tbody>
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Subjects were entering freshmen U.S. Coast Guard Academy cadets in the Classes of 1980, 1981, and 1982.

Figure 1. Frequency distribution of platelet monoamine oxidase activity for Epstein-Barr virus viral capsid antibody positive and negative cadets at entry
platelet-rich plasma was collected in acid-citrate-dextrose (ACD-A) vacutainers. Platelets were separated by differential centrifugation (Shaskan and Becker 1975a) and stored at −70°C up to 2 months. Platelet MAO activity was evaluated using 14C-tryptamine as substrate (Shaskan and Becker 1975a). Routine microbiological and clinical tests were performed by the hospital staff for the diagnosis of clinical IM.

In cooperation with the Center for Disease Control and the Yale Arbovirus Research Unit, a prospective epidemiologic study of dengue fever (DF) was carried out in the fall and winter 1977–78, during an epidemic of DF in Puerto Rico. Bloods were collected in ACD-A vacutainers and processed for platelet pellets as described above. Frozen pellets were transported in dry ice to Connecticut where platelet MAO assay was performed, using 14C-tryptamine (Shaskan and Becker 1975a).

Results

In three consecutive entering freshmen classes, 706 cadets volunteered for this study and represented a participation rate between 66 to 90 percent in these groups. As indicated in table 1, 62 percent of the cadets were EBV-VCA positive and immune to IM, and 38 percent were EBV-VCA negative and susceptible.

Blood platelet MAO activity was determined on 703 cadets at entry. MAO activity was unimodally distributed with a mean and standard deviation of 2.93 ± 0.97 nanomoles of product formed per milligram protein per hour. No significant difference was found in the mean MAO values or in the distribution of subpopulations of these cadets based upon EBV antibody status, as shown in figure 1.

Table 2. Correlation of platelet MAO values obtained from yearly blood samples

<table>
<thead>
<tr>
<th>Cadet Class</th>
<th>Pearson's r correlation coefficient yearly paired comparisons</th>
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<tbody>
<tr>
<td></td>
<td>0 vs. 1</td>
</tr>
<tr>
<td>1980</td>
<td>.65 (n = 66)</td>
</tr>
<tr>
<td>1981</td>
<td>.62 (n = 55)</td>
</tr>
<tr>
<td>1982</td>
<td>.49 (n = 84)</td>
</tr>
</tbody>
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1 p < .02; all other comparisons, p < .001.

Figure 2. Distribution of mean platelet MAO activities for susceptible cadets

Frequencies are shown for susceptible cadets who acquired the EB virus (clinical or subclinical manifestations) and cadets who have remained susceptible (EBV-VCA antibody negative).
During the first year of residence, infection rates of EB virus among susceptible cadets in the Classes of 1980, 1981, and 1982 were 10.9, 12.1 and 9.3 percent, respectively. These attack rates compare closely to the 12 percent infection rate reported in three other studies of entering college freshmen (Hallee et al. 1974). To date, 40 susceptible cadets have acquired EB virus infection demonstrated by seroconversion. Fifteen cadets were hospitalized with a diagnosis of clinical IM, but others had subclinical infections. The clinical attack rate in this population during the first year was 37.5 percent.

The mean platelet MAO activity for subjects with clinical IM was $3.10 \pm 0.71 (n = 15)$ and not significantly different from that of $2.76 \pm 0.66 (n = 25)$ for inapparent (subclinical) EBV infections (figure 2). Neither of the mean values for clinical or subclinical groups were statistically different from the mean platelet MAO activity for the remaining susceptible cadets, which was $2.95 \pm 0.87 (n = 212)$.

In order to evaluate the possible role of platelet MAO activity as a predisposing host factor in this infection, the MAO activity of seroconverters was compared to that of remaining susceptible cadets, using a $\chi^2$ analysis. Of the seroconverters, only 1 of 40 cadets (2.5 percent) had a mean platelet MAO value of less than $2.20 \text{ nanomoles/mg protein/hour}$, while 30 of 212 (14.1 percent) remaining susceptibles were found to be in this “low MAO” category ($\chi^2 = 3.223, p = .073$).

In previous investigations, Murphy et al. (1977) reported that in healthy adult males, platelet MAO levels over intervals of 1 to 2 weeks ($n = 26$) and 8 to 10 weeks ($n = 42$) were similarly correlated for both time periods ($r = .94$ and $r = .86$, respectively). As indicated in table 2, correlation coefficients ranging from .49 to .69 were found when entry MAO levels were compared to MAO values in matched plasma obtained later in 55–84 cadets. Similar correlation coefficients were found over 2 years’ samples with a $p$ value < .001, but one comparison for the Class of 1981 had an $r$ value of .35 (table 2).

Although the association of EBV antibody and platelet MAO levels suggests that acquisition of this virus infection does not have a sustained effect on platelet MAO activity, perhaps acute EBV infection, possibly through host immune mechanisms, affects it. Accordingly, we compared platelet MAO activity in matched plasma samples before and after acquisition of EBV antibodies as shown in figure 3. Platelet MAO values were available on 6 of 15 clinical IM cases before and after development of infection. Five of six individuals demonstrated decreased platelet MAO activity, but one case showed an increased MAO value (figure 3). Matched samples were also available from 19 of 25 subclinical cases; 12 of these had decreased MAO values after EBV infection as listed in figure 3.

In the comparison study of dengue virus infections in Puerto Rican
Figure 4. Frequency distribution of platelet monoamine oxidase activity in Puerto Rican males

\[ \bar{X} = 3.15 \pm 0.96 \]
\[ N = 222 \]

PLATELET MAO ACTIVITY
(nMOLES/mg PROTEIN/HR)

Discussion

Among 706 freshmen cadets entering the U. S. Coast Guard Academy, 38 percent lacked detectable EBV antibody in serum samples and were susceptible to IM. This compares to 36 percent susceptibles in a population of 1,401 cadets studied earlier at the U. S. Military Academy, West Point (Hallee et al. 1974). The yearly EBV infection rate ranged from 9.3 to 12.1 percent and these compared to a
rate of 12.3 percent at West Point (Halle et al. 1974). In the present study 37.5 percent of subjects acquiring EBV infection developed clinical infectious mononucleosis. This is similar to a clinical attack rate of 27.7 percent observed among West Point cadets (Halle et al. 1974) but considerably lower than rates of 59.1 percent in five English schools (Joint Investigation by University Health Physician and P.H.L.S. Laboratories 1971) and 74.0 percent among Yale University freshmen (Sawyer et al. 1971). This variation in clinical attack rates by the Epstein-Barr virus assumes sociobiological significance in that the seroconversion rates in each of these populations were remarkably similar, i.e., approximately 12 percent.

Clinical attack rates may be influenced by differences in motivation of students to seek medical care and by differences in intensity of clinical surveillance. Since IM is not a mild illness and since cadets are required to participate in all daily physical and academic activities or have a medical excuse, it is unlikely that these differences contribute significantly to the lower clinical attack rates at the service academies. Perhaps psychosocial and biochemical host factors are relevant variables influencing clinical attack rates. For instance, in a recent study of 1,401 West Point Cadets, a high level of academic motivation was found to be associated with increased rates of clinical IM among subjects demonstrating EBV seroconversion (Kasl, Evans, and Niederman, 1979). Might platelet MAO activity be a host factor?

Platelet MAO activity of 703 entering cadets was unimodally distributed. No significant differences were found in comparison of means and/or distributions of MAO levels in subjects with or without EBV antibody; thus previous Epstein-Barr infections were not found to influence platelet MAO activity (figure 1). However, the observations suggest that EBV infections may exert transient alterations in platelet MAO activity as shown in figure 3. Five of six clinically symptomatic patients displayed decreasing platelet MAO values. Nevertheless, platelet MAO activity does not exert a directly precipitating effect in infectious mononucleosis, as depicted by comparative MAO values for clinical and subclinical cases of IM in figure 2. However, platelet MAO activity may have a predisposing influence, since only 1 of 40 cadets who developed EBV seroconversion had a mean platelet MAO value less than 2.20 nanomoles/mg protein/hour (figure 2).

Although production of EBV capsid antibody is a function of the host's humoral immune system, there is now evidence to support the notion that cell-mediated immune responses are also involved in this infection (Evans and Niederman 1976; Mangi et al. 1974). Transient
depression of delayed type hypersensitivity has been reported during acute infectious mononucleosis (Bentzon 1953) and alterations of T-lymphocytes have also been reported (Sheldon et al. 1973; Tosato et al. 1979).

In contrast, another viral infection, dengue fever, is a disease in which the host's immune response is predominantly humoral, involving circulating antibody and the complement system (Downs 1976). The distribution of platelet MAO activity in healthy Puerto Rican males was similar to the distribution in Coast Guard cadets, and no apparent difference developed in platelet MAO activity values in cases having clinical or subclinical dengue, compared to noninfected subjects. Thus, platelet MAO activity does not seem to be a host factor in dengue fever, although it may have some role in cell-mediated immune responses in infectious mononucleosis.

As a host factor, there are at least three possible biological correlations of platelet MAO activity: first, it could reflect brain MAO activity; second, as an index of brain MAO activity, platelet MAO may reflect the synaptic disposition of a variety of neurotransmitter or neuromodulator amines, notably those which have high affinity for the B-form of the enzyme, such as phenylethylamine, tyramine, octopamine, tryptamine, and dopamine (Neff and Goridis 1972); third, platelet MAO activity may reflect lymphocyte or microglial MAO activity (Sullivan et al. 1978), as lymphocytes, monocytes, and platelets have a common origin from hematopoietic stem cells (Quensenberry and Levitt 1979) and microglial cells have monocytes as their initial precursors (Imamoto and Leblond 1978). Given the spectrum of these relationships, possible mechanisms by which platelet MAO activity represents a host factor in disease might include MAO activity in brain and immune system, or both, affecting cellular immune responsiveness.

Does the suggestive evidence that platelet MAO activity may play a predisposing role in a cell-mediated infectious disease have any relevance to the pathogenesis of schizophrenia? Retrospective clinical studies of this disease (reviewed by Wyatt, Potkin, and Murphy 1979) have reported a higher incidence of low platelet MAO activity individuals among schizophrenics and patients with other psychiatric disorders. Family and twin studies of schizophrenia (Wyatt et al. 1973), bipolar affective disorders (Leckman et al. 1977) and alcoholism (Sullivan et al. 1979) support the notion that low platelet MAO activity is a genetically determined trait that could be a risk factor for psychiatric illness. Recent prospective studies of college student populations have suggested that low platelet MAO activity predisposes to psychiatric illness (Buchsbaum, Coursey, and Murphy 1976). Platelet MAO activity has been observed to be reduced in both platelets and circulating blood lymphocytes in chronic schizophrenic patients, whereas enzyme activity in acute schizophrenic cases was similar to that in control subjects (Sullivan et al. 1978). It will be necessary to know if this MAO activity is similarly reduced in both T- and B-lymphocytes in relation to studies of the immunology of schizophrenia (Bergsma and Goldstein 1978). It is clear that prospective clinical-epidemiologic studies will be required to evaluate the status of low platelet MAO activity as a host factor which may alternatively influence the incidence of psychiatric and somatic disease.

References


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