Primary Human Herpesvirus 8 Infection in Immunocompetent Children

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Context Human herpesvirus 8 (HHV-8) infection causes Kaposi sarcoma and lymphoproliferative disorders in immunosuppressed adults. Its manifestations in immunocompetent hosts are unknown.

Objectives To determine whether HHV-8 primary infection is symptomatic in immunocompetent children and to identify the epidemiological and virological correlates of HHV-8 infection.

Design and Setting Prospective cohort study conducted in the pediatric emergency department of a hospital in Alexandria, Egypt, between December 1, 1999, and April 30, 2000.

Patients Eighty-six children aged 1 to 4 years who were evaluated for a febrile syndrome of undetermined origin.

Main Outcome Measures Serological assay and polymerase chain reaction of blood and saliva samples for HHV-8. Information on potential risk factors for HHV-8 infection was also collected.

Results Thirty-six children (41.9%) were seropositive; HHV-8 DNA sequences were detected in 14 (38.9%) of these 36 children (detected in saliva in 11 of 14). Significant associations were found between HHV-8 infection and close contact with at least 2 other children in the community (36 of 63 vs 6 of 23 for <2 children; adjusted odds ratio [OR], 3.50; 95% confidence interval [CI], 1.11-12.22) and admission to the emergency department in December or January (28 of 47 vs 14 of 39 for February-April; adjusted OR, 3.15; 95% CI, 1.23-8.58). Six children had suspected primary HHV-8 infection; all but 1 had a febrile cutaneous craniocaudal maculopapular rash, which was more common among these children (5 of 6 vs 10 of 75; P < .001). For 3 of these 6 children, a second blood sample was obtained after the convalescence phase, and all 3 seroconverted for HHV-8.

Conclusions Primary infection with HHV-8 may be associated with a febrile maculopapular skin rash among immunocompetent children. The finding of HHV-8 DNA sequences in saliva supports the hypothesis that transmission through saliva is the main mode of transmission in the pediatric age group.

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HERPESVIRUS 8 INFECTION IN IMMUNOCOMPETENT CHILDREN

patient and with bone marrow failure with plasmocytosis in the other.13

The objectives of this study were to
determine whether primary HHV-8 in-
fec tion can cause specific clinical mani-
festations in immunocompetent chil-
dren and to identify the epidemiological
and virological correlates of HHV-8 in-
fec tion.

METHODS
Study Population and Design
Study participants were children con-
secutively treated at the pediatric emer-
gency department of the Medical Re-
search Institute, University of Alexandria,
Egypt, between December 1, 1999, and
April 30, 2000. During the study pe-
riod, 486 children who were aged 1 to 4
years and had an acute febrile illness
(temperature, ≥38°C) of undeter-
mined origin were evaluated. Of these,
130 (26.7%) were excluded because of
a clinical or serological diagnosis of a
specific exanthematous illness (ie, measles
[42 cases], chickenpox [32 cases], and
rubella [25 cases]) or a diagnosis of
mumps (10 cases) or influenza (21
cases). Informed consent was obtained
for 110 of the remaining 356 children
(30.9%). Most parents (231) who re-
fused to have their children.

The study was approved by the Medi-
cal Research Institute of Alexandria,
Egypt, and conducted as part of the
Italo-Egyptian Cooperation Project.
Written informed consent was ob-
tained from the children’s parents. The
principles outlined in the Declaration
of Helsinki were followed.14

When children arrived, blood and sa-
liva samples were collected. To detect
HHV-8 infection, we performed poly-
merase chain reaction (PCR) tests on
plasma and saliva and serological as-
say on plasma; the 2 tests are de-
scribed below. A child was considered
infected with HHV-8 if the result of at
least 1 of these tests was positive.

Children whose PCR test results were
positive yet who had negative serologi-
test results were assumed to have pri-
mary HHV-8 infection, and they were
traced to repeat the serological assay.

Using standard forms, we inter-
viewed parents about the following po-
tential risk factors for HHV-8 infec-
tion: age, sex, place of residence
(Alexandria vs rural settings), number
of siblings, number of children in the
community with whom the child
had had close contact in the previous
3 weeks (≥2 vs <2 children), contact
with domestic animals, current symp-
toms and their duration, and the oc-
currence of other diseases in the 12
months before emergency department
treatment. No drugs other than anti-
pyretics or antibiotics were adminis-
tered to the children.

Detection of HHV-8 by PCR
To detect HHV-8 in DNA in plasma and
saliva, we used a nested PCR that was
optimized to reproducibly detect an in-
put of 1 to 5 DNA copies. The first-
round primers, described by Lock et
al,15 produced a 300–base pair frag-
ment that was then amplified with
nested primers1 to produce a 233–
base pair fragment derived from the
HHV-8 open reading frame 26 region.
The DNA was extracted from 200 µL
of plasma or saliva by a High Pure PCR
template preparation kit (Roche Diag-
nostics GmbH, Mannheim, Ger-
many). Each PCR reaction contained
100 ng of each primer, 2 mM of mag-
nesium chloride, 187.5 µM of each
nucleotide (dNTP), 1 unit of Taq DNA
polymerase (Promega Corporation,
Madison, Wis), and 0.2 to 0.5 µg of tar-
get DNA in ammonium dichloride
buffer, for a final volume of 50 µL. The
cycle characteristics used were identi-
fic for both rounds: hot start (95°C) for
6 minutes followed by 39 cycles of de-
naturation at 94°C for 30 seconds, an-
nealing at 60°C for 30 seconds, and ex-
tension at 72°C for 30 seconds. The final
extension was increased to 5 minutes.

The PCR amplicons were detected by
electrophoresis of 10 µL of the ampli-
fied reaction, plus loading buffer, against a marker of appropriate mo-
lecular weight on a 3% agarose gel.

All the samples were analyzed in a
blinded fashion, and the results were
confirmed by repeated assays, al-
though the results of all of the re-
peate de assays are not presented here.
To identify false-positive reactions,
specimens were processed in parallel
with control samples.

Serological Assays
To detect antibodies to lytic antigens
of HHV-8, we used an immunoflu-
orescent assay based on the BCBL-1 cell
line (obtained from M. McGrath, MD,
and D. Ganem, MD, through the
Acquired Immunodeficiency Syn-
drome Research and Reference
Reagent Program, Division of AIDS,
National Institute of Allergy and Infec-
tious Diseases, National Institutes of
Health). A detailed description of the
methods used has been reported else-
where.7,9 Samples reactive at 1:20 dilu-
tion in the antilytic test were consid-
ered positive. Serum samples were
also tested to detect antibodies to
latent antigens of HHV-8 by an immu-
nofluorescent assay based on the
BCP-1 cell line (obtained from Denise
Whitby, PhD, National Cancer Insti-
tute, Rockville, Md). This assay was
described by Gao et al,3 and samples
were scored as positive if they pro-
duced punctate nuclear staining at a
serum dilution of 1:100, whereas any
homogeneous cytoplasmic staining
was ignored. All microscopic examina-
tions were done under code in a
blinded fashion. The combination of
the 2 immunofluorescent assays
showed the best sensitivity and speci-
ficity (89.1% and 94.9%, respectively)
in a multicenter study.16

We also determined the occurrence
of infection with other ubiquitous her-
pesviruses. Specifically, we tested for
the presence of antibodies to cytomegalo-
ivirus (HHV-5), Epstein-Barr virus
capsid antigen, and human her-
pesvirus 6 (HHV-6) by using immuno-

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enzymatic assays (respectively, cytomegalovirus IgG, viral cap antigen IgG [Gull Laboratories Inc, Salt Lake City, Utah], and Milenia HHV-6 IgG [Diagnostic Products Corporation, Los Angeles, Calif]).

**Statistical Analysis**

We used the Fisher exact test to evaluate the association between clinical manifestations and HHV-8 infection, conducting separate analyses according to the presence of HHV-8 antibodies, DNA sequences, or both. Associations with a P<.017 were considered significant according to the conservative Bonferroni inequality adjustment for multiple comparisons.

We assessed the relationship between antilytic HHV-8 antibodies and PCR by calculating the proportion of PCR-positive individuals by level of antilytic titer (ie, 1:20, 1:40, 1:80, 1:160, 1:320, and 1:640). The statistical significance of the association was assessed by the χ² test for trend, and associations with a P<.05 were considered significant.

We assessed the association between potential risk factors and HHV-8 infection by estimating crude and adjusted odds ratios (ORs) and their 95% confidence intervals (CIs) through univariate and multivariate logistic models. We tested for interactions and multicollinearity by studying the correlation between each couple of predictor variables. In the multivariate analysis, we considered only sex and those variables that were significantly associated with HHV-8 infection in the univariate analysis. Associations with a P<.05 were considered significant. STATA version 6 (STATA Press, College Station, Tex) was the statistical software used.

**RESULTS**

We enrolled 86 children with a febrile syndrome of undetermined origin. Their median age was 36 months (range, 12-46 months). Forty-eight children (55.8%) were males. Of the 86 children, 33 (38.4%) presented with cough or other respiratory tract symptoms, 24 (27.9%) presented with a rash, 14 (16.3%) with painful oral ulcers, 13 (15.1%) with no symptoms, 10 (11.6%) with watery diarrhea, 9 (10.5%) with seizures, and 3 (3.5%) with middle ear infection.

**HHV-8 Serological and Virological Findings**

Thirty-six children (41.9%) had antilytic antibodies to HHV-8 when treated at the emergency department. For 14 of these 36 children (38.9%), HHV-8 DNA sequences were detected in saliva or plasma (TABLE 1): HHV-8 sequences were detected in the saliva of 11 children (30.6%), and for 2 of these 11 children (5.6% of the 36 children), sequences were also detected in plasma. For the remaining 3 patients (8.3%), HHV-8 DNA sequences were detected only in plasma. For 5 children, all of whom were HHV-8 seropositive, samples for PCR were unavailable.

Twelve of the 86 children (14.0%) had antibodies against HHV-8 latent antigens, all of them with a positive antilytic pattern.

Herpesvirus 8 DNA sequences were detected in 6 (12.0%) of the 50 HHV-8–seronegative children, all of whom had HHV-8 DNA sequences in saliva (2 of these 6 children also had sequences in plasma).

**Demographic and Environmental Correlates of HHV-8 Infection**

The associations between HHV-8 infection (defined as seropositivity, detection of HHV-8 DNA sequences, or both) and certain demographic and environmental characteristics are shown in TABLE 2. The results of the univariate analysis showed no significant association with sex, age, place of residence (ie, Alexandria vs rural settings), contact with domestic animals, the mean duration of symptoms before treatment at the emergency department, or the occurrence of other diseases in the previous 12 months. In the univariate analysis, significant associations were found for patients having siblings, for close contact with at least 2 children in the community in the previous 3 weeks (compared with fewer than 2 children), and for having been treated at the emergency department in December or January (compared with February through April). The results of the multivariate analysis showed that the associations remained significant for close contact with at least 2 children in the community and for treatment in December through January, whereas having siblings no longer showed a significant association.

**Clinical Correlates of HHV-8 Infection**

Certain demographic characteristics and the clinical manifestations of the 81 children for whom samples for PCR were available are shown in TABLE 3, according to the results of the serological assay and PCR. Of the 50 children who had no HHV-8 antibodies at enrollment, 6 had HHV-8 DNA sequences (all 6 in saliva and 2 in both saliva and plasma), suggesting that these children had primary HHV-8 infection. These children were between 24 and 36 months of age, and all of them had visited the emergency department in December or January. All but 1 of these 6 children presented with a cutaneous maculopapular rash, which was significantly (P<.001) more common among these children than among the other groups of children (defined

| Table 1. Detection of Human Herpesvirus 8 (HHV-8) DNA Among HHV-8–Seropositive and HHV-8–Seronegative Children* |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| HHV-8 Antilytic Antibodies | Positive/Negative | Positive/Positive | Negative/Negative | Negative/Positive | Total |
| Positive | 9 | 2 | 3 | 17 | 31 |
| Negative | 4 | 2 | 0 | 44 | 50 |
| Total | 13 | 4 | 3 | 61 | 81 |

*Samples were unavailable for 5 children who were positive for HHV-8.
according to the results of the serological assay and PCR). The rash first appeared on the face and gradually spread to the trunk, arms, and legs. It initially consisted of discrete red macules that blanched with pressure and became papular. The median duration of the rash was 6 days (range, 3-8 d). In the 6 children, the fever persisted for a median of 10 days (range, 2-14 d), and 4 of them had a high-grade fever (temperature, ≥39°C). An upper respiratory tract infection appeared as a secondary symptom in 5 of these 6 children, and a lower respiratory tract infection appeared as a secondary symptom in 2 of the 6 children; major respiratory complications did not occur during the course of HHV-8 infection. Neither lymphadenopathy nor oral ulcers were detected in these 6 children. The child who did not have a rash (a 24-month-old boy) experienced confusion, convulsions, and vomiting 48 hours after the onset of persistent high-grade fever. All 6 children showed a good clinical outcome.

To confirm the occurrence of primary HHV-8 infection in these 6 children.

### Table 2. Associations Between Human Herpesvirus 8 (HHV-8) Infection and Demographic and Environmental Characteristics in 86 Children

<table>
<thead>
<tr>
<th></th>
<th>HHV-8 Infection, No./Total (%)</th>
<th>Univariate Analysis</th>
<th>Multivariate Analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OR (95% CI)</td>
<td>P Value</td>
<td>OR (95% CI)</td>
</tr>
<tr>
<td>Age, median (range), mo</td>
<td>36 (12-46)</td>
<td>1.05 (0.92-1.37)</td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>16/38 (42.1)</td>
<td>1.63 (0.69-3.88)</td>
<td>.27</td>
</tr>
<tr>
<td>Male</td>
<td>26/48 (54.2)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Month emergency department visited</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>December-January</td>
<td>28/47 (59.6)</td>
<td>2.63 (1.11-6.45)</td>
<td>.03</td>
</tr>
<tr>
<td>February-April</td>
<td>14/39 (35.9)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Contact with other children, No. ≥2</td>
<td>36/63 (57.1)</td>
<td>3.78 (1.37-11.66)</td>
<td>.01</td>
</tr>
<tr>
<td>Contact with other children, No. &lt;2</td>
<td>6/23 (26.1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Siblings</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>27/43 (62.8)</td>
<td>3.15 (1.33-7.77)</td>
<td>.01</td>
</tr>
<tr>
<td>No</td>
<td>15/43 (34.9)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Place of residence</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rural area</td>
<td>35/67 (52.2)</td>
<td>2.19 (1.20-48.37)</td>
<td>.53</td>
</tr>
<tr>
<td>Missing information</td>
<td>6/16 (37.5)</td>
<td>1.20 (0.09-29.14)</td>
<td>.89</td>
</tr>
<tr>
<td>Urban area (Alexandria)</td>
<td>1/3 (33.3)</td>
<td>1.02 (0.37-2.94)</td>
<td>.65</td>
</tr>
<tr>
<td>Symptoms duration, median (range), d</td>
<td>10 (2-14)</td>
<td>1.20 (0.52-2.84)</td>
<td>.45</td>
</tr>
<tr>
<td>Occurrence of other disease in the previous 12 mo</td>
<td>17/42 (40.5)</td>
<td>1.20 (0.46-3.16)</td>
<td>.17</td>
</tr>
<tr>
<td>Contact with animals</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>13/33 (39.4)</td>
<td>1.86 (0.78-4.57)</td>
<td>.17</td>
</tr>
<tr>
<td>Yes</td>
<td>29/53 (54.7)</td>
<td></td>
<td></td>
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</tbody>
</table>

*Infection was defined as seropositive, detection of HHV-8 DNA sequences, or both. OR, indicates odds ratio; CI, confidence interval.

### Table 3. Serological Assay and Polymerase Chain Reaction (PCR) for Human Herpesvirus 8 (HHV-8) Infection in 81 Children

<table>
<thead>
<tr>
<th>Demographic Characteristics and Clinical Manifestations</th>
<th>Results of HHV-8 Serological Assay and PCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seronegative and PCR-Positive (n = 8)</td>
<td>Seropositive and PCR-Positive (n = 14)</td>
</tr>
<tr>
<td>Age, median (range), mo</td>
<td>36 (24-36)</td>
</tr>
<tr>
<td>Sex, male/female</td>
<td>4/2</td>
</tr>
<tr>
<td>Admission in December or January, No. (%)</td>
<td>6 (100)</td>
</tr>
<tr>
<td>Fever type, No. (%)*</td>
<td>Low-grade</td>
</tr>
<tr>
<td>High-grade</td>
<td>2 (33.3)</td>
</tr>
<tr>
<td>Persistent high-grade</td>
<td>2 (33.3)</td>
</tr>
<tr>
<td>Watery diarrhea, No. (%)</td>
<td>1 (16.6)</td>
</tr>
<tr>
<td>Rash, No. (%)†</td>
<td>Maculopapular</td>
</tr>
<tr>
<td>Hemorrhagic</td>
<td>0</td>
</tr>
<tr>
<td>Other</td>
<td>0</td>
</tr>
<tr>
<td>P value</td>
<td>.01</td>
</tr>
<tr>
<td>Cough, No. (%)</td>
<td>2 (33.3)</td>
</tr>
<tr>
<td>Sore throat, No. (%)</td>
<td>5 (83.3)</td>
</tr>
<tr>
<td>Painful oral ulcers, No. (%)</td>
<td>0</td>
</tr>
<tr>
<td>Cervical lymphadenopathy, No. (%)</td>
<td>0</td>
</tr>
<tr>
<td>Convulsion, No. (%)</td>
<td>1 (16.7)</td>
</tr>
</tbody>
</table>

*Low-grade fever indicates a temperature of 38°C-38.9°C; high grade fever, ≥39°C; and persistent high-grade fever, lasting more than 72 hours.
†The Fisher exact test was used for P values; seronegative and PCR-positive was the reference group.
dren, we obtained additional blood samples 6 months after acute infection and repeated the serological assay. We succeeded in obtaining samples from 3 of these children, all of whom had a maculopapular rash when treated at the emergency department. All 3 children had undergone seroconversion; the antibody titer for antilytic antigen was 1:80 for 2 children and 1:160 for 1 child. This patient also had an antilatent antibody titer of 1:200.

Antibodies (IgG) to cytomegalovirus, Epstein-Barr virus, and HHV-6 were detected in 89%, 83%, and 68% of the entire study population, respectively. The percentages of children with antibodies to these viruses did not significantly differ among the groups defined, according to the results of the serological assay and PCR (data not shown). All 3 of the children who had undergone seroconversion for HHV-8 had antibodies to Epstein-Barr virus at the baseline sample; 1 had a negative HHV-6 test result and another had a negative cytomegalovirus test result; both also had negative test results at the follow-up sample.

**COMMENT**

Our results suggest that primary HHV-8 infection can be symptomatic in immunocompetent persons. Five of the 6 children with suspected primary HHV-8 infection had a prolonged craniocaudal maculopapular rash. The other symptoms in these children included high-grade fever (4 of the 6 children) and generalized involvement of pharyngeal mucosa, with no oral ulcers or reactive cervical or submandibular lymphadenopathy. The child without a rash experienced convulsions and vomiting, which occurred 48 hours after the onset of the first clinical symptoms. All of the children recovered completely within 3 weeks of their treatment at the emergency department.

Two other children with detectable DNA sequences had a clinical picture similar to that described for the children with suspected HHV-8 infection (ie, maculopapular rash without lymphadenopathy). Although we did not suspect that these 2 children had primary infection because their test results were seropositive, they both had a low antibody titer (ie, 1:40), which suggests that they may have had primary infection. Febrile craniocaudal maculopapular rash was also observed in 8 other children, none of whom had detectable HHV-8 sequences (5 were also HHV-8 seronegative and 3 were HHV-8 seropositive); however, the overall clinical picture for these children differed from that of the children with suspected primary infection because all 8 of these children had cervical lymphadenopathy and oral ulcers. The high proportion of children with HHV-8 primary infection is not surprising, considering the high seroprevalence found in Egyptian population groups in a previous study, suggesting a high viral circulation in early childhood.

There have been only 3 reports of primary HHV-8 infection associated with specific symptoms, yet all 3 patients had severe immunosuppression. Febrile, cutaneous maculopapular rash, and hepatitis were recently reported in a patient with non-Hodgkin lymphoma after autologous peripheral blood stem cell transplantation associated with the reactivation of HHV-8 infection. To the best of our knowledge, this study is the first report of symptomatic primary HHV-8 infection in immunocompetent persons. Before conclusions are drawn from our results, some limitations need to be discussed. First, we succeeded in collecting sequential serum samples for only 3 of the 6 children with suspected primary infection. However, all 3 of these children underwent seroconversion, confirming that the combination of negative serology and positive PCR is suggestive of primary infection and may represent a reliable marker of HHV-8 seroconversion. During primary HHV-8 infection, the time between virus replication and the appearance of antibodies is variable (ie, 3-12 months). This timing is similar to that of other primary viral infections, such as HHV-6. In the first days of illness, the detection of viremia by coculture or molecular methods is unassociated with the presence of specific antibodies. Second, the antibody titer of the second serum samples was relatively low, possibly because of the lag time between the acute episode and blood collection (approximately 6 months). However, the sequence of events (ie, PCR positivity during the acute episode followed by seroconversion in all 3 children) strongly supports the hypothesis of a causal association. Third, the selection of children with an acute febrile illness that was serious enough to warrant treatment at an emergency department could represent a bias toward the identification of more severe cases. Fourth, the relatively small subgroup sample size was reflected in wide CIs for the ORs, limiting the precision of the estimate of the associations. Finally, the low participation rate means that these 2 children had primary infection because their test results were seropositive, they both had a low antibody titer (ie, 1:40), which suggests that they may have had primary infection. Febrile craniocaudal maculopapular rash was also observed in 8 other children, none of whom had detectable HHV-8 sequences (5 were also HHV-8 seronegative and 3 were HHV-8 seropositive); however, the overall clinical picture for these children differed from that of the children with suspected primary infection because all 8 of these children had cervical lymphadenopathy and oral ulcers. The high proportion of children with HHV-8 primary infection is not surprising, considering the high seroprevalence found in Egyptian population groups in a previous study, suggesting a high viral circulation in early childhood.

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Because HHV-8 infection was detected in more than 40% of children in the study, the disease prevalence may be high among Egyptian children with acute febrile illness. The lower prevalence of antilatent antibodies may be explained by low sensitivity of this assay. The HHV-8 DNA sequences detected in the saliva of approximately 30% of the seropositive children indicate that HHV-8 shedding in the saliva is relatively common. This finding provides biological plausibility to the hypothesis of horizontal transmission among children, which has been proposed by epidemiological studies. This hypothesis is also supported by our finding of an association between HHV-8 infection and close contact with other children and having siblings.

**Author Contributions:** Study concept and design: Andreoni, Parisi, Rezza. Acquisition of data: El Sawaf, El Zalabani, Uccella, Bugarini. Analysis and interpretation of data: Andreoni, Sarmati, Nicastri, Bugarini, Rezza. Drafting of the manuscript: Andreoni, Sarmati, Nicastri, El Sawaf, El Zalabani, Uccella, Bugarini. Critical revision of the manuscript for important intellectual content: Andreoni, Parisi, Rezza. Statistical expertise: Bugarini, Rezza. Obtained funding: Andreoni.
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Administrative, technical, or material support: Sarmati, Nicastrì, El Sawaf, El Zalabani, Uccella. Study supervision: Andreoni, Sarmati, El Sawaf, Pa-risi, Rezza.

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REFERENCES

Of all our possessions wisdom alone is immortal.
—Isocrates (436-338 BCE)