Pro- *versus* anti-inflammatory cytokine profile in African children with acute oro-facial noma (*cancrum oris*, noma)

Reshma S. Phillips[1], Cyril O. Enwonwu[1,2], William A. Falkler[1]

[1] Department of Biomedical Sciences, School of Dentistry, University of Maryland, 666 West Baltimore St, Rm 4G31, Baltimore, MD 21201, USA
[2] Department of Biochemistry and Molecular Biology, School of Medicine, University of Maryland, Baltimore, Maryland, USA

Correspondence : Cyril O. Enwonwu
<Oyagom@aol.om>

ABSTRACT. Fresh noma is a severe orofacial necrosis with an astonishingly rapid development. It is seen mainly in malnourished children less than 4 years old from developing countries. Cytokines play a central role in oral mucosal inflammation. We therefore studied the relevance of circulating cytokines to noma, and the key microorganisms associated with the lesion. Nigerian village children with acute noma (n=68) and their neighborhood village (n=63) as well as urban (n=45) counterparts of comparable age and free of overt infections were evaluated for serum cytokine levels by ELISA. Oral bacteria were studied by polymerase chain reaction. Evaluation of random cases of the village and noma children showed marked depletion ($p<0.05$ or 0.001) of the plasma antioxidant micronutrients (retinol, ascorbic acid, zinc) as well as albumin and blood hemoglobin in the latter, relative to the former group. Concentrations of the circulating, pro-inflammatory cytokines (IL-18, IL-6, IL-12, IL-8, IFN-γ) and the soluble inhibitors (TNFR-p55, TNFR-p75 and IL-1ra) were significantly higher ($p< 0.01$ or 0.001) in noma children than in the healthy urban children, but less so when compared to their neighborhood village counterparts. The increase in levels of the anti-inflammatory/regulatory cytokines (IL-4, IL-10 and TGF-β) was less marked relative to the pro-inflammatory cytokines. Bacteria observed at the highest frequencies in noma lesions were *P. intermedia* (83%), *T. forsythensis* (83%), *P. gingivalis* (50%), *C. rectus* (50%) and *T. denticola* (50%). We conclude that noma is an immunopathological response to potent bacterial factors resulting in uncontrolled production of cytokines and possibly other, still unknown, inflammatory mediators.

Keywords: noma, cytokines, infections, malnutrition, oral sepsis, cancrum oris

Noma (cancrum oris), a debilitating oro-facial gangrene, occurs in several regions of the world, but with notable prevalence in sub-Saharan Africa [1, 2]. Fresh noma is most frequently seen in children ages 1 to 4 years, although adolescents and adults are not exempt from recurrent or late stages of the disease [3, 4]. The lesion begins as a localized ulceration of the buccal mucosa and spreads very rapidly through the oro-facial tissues, establishing itself as a gangrenous area with a blackened necrotic center surrounded by a well-demarcated groove [5, 6]. The environment in which the disease thrives is characterized by extreme poverty [7, 8], severe malnutrition, unsafe drinking water, deplorable sanitary practices, residential proximity to unkempt domestic animals, poor oral health practices, and a high prevalence of endemic diseases such as measles, malaria, diarrhea and tuberculosis [9-11]. Children reared under such hostile conditions are reported to be victims of infection-induced chronic and recurrent immunostimulation by environmental antigens [12]. In these deprived communities, exclusive breast-feeding is not practiced in the first few months of life. Thus, the child's growth velocity falls markedly from about 3-4 months of age following introduction of contaminated, poor quality weaning foods, and continues until about the age of 36 months [13, 14]. These stunted children often show evidence of chronic, cell-mediated enteropathy, with intestinal mucosal cytokine production biased towards a pro-inflammatory response [14]. Malnutrition is believed to account for no more than 40% of the variance in the occurrence of stunting, which is attributed mainly to a continuous burden of infections [15]. On first encounter, Nigerian children with fresh, acute noma usually present with fluctuating fever varying between 101 and 105 °F (38.3 - 40.5 °C), tachycardia, markedly increased respiratory rate, severe microcytic anemia, pulmonary involvement and white blood cell counts often as high as 20,000 to 30,000 cells/mm^3. These are manifestations of sepsis or the systemic inflammatory response to infection. There is, as yet, no specific microorganism incriminated in the causation of noma [10, 16, 17].

Noma is not seen in children of elite Nigerians residing in affluent sections of the urban areas. It is rather a socioeconomic disease afflicting preferentially the deprived, malnourished children in the poor, rural communities [1]. Significantly elevated ($p<0.01$) plasma concentrations of interleukin (IL)-6, C-reactive protein (CRP) and the soluble receptors of tumor necrosis factor-α (sTNFR-p55 and sTNFR-p75) have been reported in malnourished Afri-

can children with compromised antioxidant status compared with their healthy control counterparts, and in both groups, infections elicit elevations in levels of the inflammatory mediators compared to the non-infected individuals [18]. Pro-inflammatory cytokines are necessary for initiating an effective inflammatory process against infection, while the anti-inflammatory cytokines regulate the inflammatory response [19]. We therefore hypothesized that noma results from chronic immunostimulation by oral microorganisms (both commensal and/or nonresident newcomers) in severely malnourished children, and may be due to excessive or inappropriate production of cytokines. In effect, the pathogenesis of noma is related to an imbalance between pro- and anti-inflammatory cytokines, the problems with this simplistic classification of inflammatory cytokines notwithstanding [20]. We measured serum levels of several pro- and anti-inflammatory cytokines in deprived, Nigerian rural children with incident noma, and in elite, Nigerian urban-based children of comparable age without noma. We also included as an extra "control" group, impoverished malnourished children of comparable age without noma and recruited from the same rural communities as the noma victims.

PATIENTS AND METHODS

Ethical considerations

This study was carried out with the approval of the Institutional Review Board (IRB) of the University of Maryland School of Medicine and the Ministry of Health in Sokoto State, Nigeria. The project was classified as high risk by the IRB. Village chiefs in charge of the relevant rural communities gave their consent to the study. Informed consent was obtained from the children's parents or legal guardians, usually in the presence of a neutral primary healthcare worker, and in all cases, the child's dissent prevailed over parental permission. Children who refused to participate in the study were treated for their health problems at the Sokoto State Hospitals, including the specialized Noma Children Hospital.

Site of study: the major site of the study was the Noma Children Hospital, a 60-bed hospital established in 1999 in Sokoto State, North-Western Nigeria. An average of 55 to 75 new cases of noma are seen annually in this hospital. Details about the demography of Sokoto State have been reported [9]. Indigenes of the rural communities generally resided in over-crowded, poorly ventilated mud huts with thatched bamboo roofs and dirt floors [11]. They often shared the unhygienic living facilities, in very close proximity with their domestic animals, e.g. cows, donkeys, rams and goats. Drinking water was obtained mainly from contaminated, shallow wells. Facilities for safe disposal of human and animal fecal wastes were grossly inadequate.

The principal health problems in the communities were malnutrition and infections such as malaria, measles, tuberculosis, pneumonia and diarrhea [11]. Prevalence of low birth weight in such rural Northern Nigerian villages is about 20% [21]. Infant mortality rate was estimated to be 114/1,000 live births, and mortality among children less than 5 years was as high as 300/1,000 live births in some communities. Exclusive breast feeding in the first 3 months of life was extremely rare in the villages. Supplementary foods given to the infants included locally obtained unpasteurized cow's milk, glucose water, herbal tea, and various indigenous cereal-based diets prepared under less than adequate hygienic conditions. Immunization coverage against measles and other childhood diseases was very low.

Subjects

Over a period of 4 years (1999-2003), our field team, which included physicians, dentists, and other health personnel, actively sought noma cases through house visits in various local government areas. Only fresh, incident noma cases were recruited into this study although the precise duration of each lesion could not be ascertained. The differential diagnosis of acute noma included exclusion of "noma neonatorum" which affects newborn and usually preterm infants, and ulcerative lesions such as leishmaniasis, agranulocytic angina, malignant oral lesions, and some manifestations of syphilis [1, 4, 22]. Other exclusion criteria included congenital abnormalities, therapy with steroids or antibiotics or traditional medications within the preceding 48 hours, concurrent presence of other overt infectious diseases particularly measles and clinically evident malaria, and subsequent serological confirmation of the presence of HIV-infection. Two control groups of comparable ages as the noma children were recruited into the study during the same time period. The first control group, referred to as the "Neighborhood Village Control" included children with no overt clinical infections, and were from the same socioeconomically-deprived, malnourished, rural communities as the noma victims. Children in this group were not necessarily in good health and the presence of sub-clinical infections, particularly intestinal parasitism, could not be ruled out. In effect, these neighborhood children were potentially at risk for noma. The second control group (Urban Elite Control) consisted of Nigerian children from educated, elite families resident in the relatively affluent sections of the cities. These control groups were children attending out-patient clinics and primary health care centers for routine checks, and had no recent history of any disease, fever and diarrhea, nor were they on any medication.

Age was determined from birth records where available, and from interviews with mothers/legal guardians, using a validated local calendar of important ceremonial/cultural events occurring in recent months/years as a guide. In a few instances, the eruption status of primary teeth in Nigerian children was used as an additional guide [23].

Biochemical studies

Venous blood was collected into plain and heparinized tubes from each subject between 8:00 am and 10:00 am. Because of the high-risk nature of the study, no more than 5 mL of blood was collected from each child, and only at one encounter. This was a constraint on the number of cytokines that could be analyzed for each sick child enrolled in the study. Care was taken to protect the blood samples from undue exposure to light, heat, and air. The samples were centrifuged (2,000 x g for 10 min), usually within 30 minutes after collection. Hemolyzed samples

were discarded and most of such samples were from the malnourished groups, particularly the children with noma. The separated plasma and serum were divided into aliquots for storage at -70 °C and subsequent analysis. The assays were carried out within one to three months of blood collection.

Assay of interleukins

Serum cytokine levels were measured in duplicate using ELISA/EASIA (enzyme amplified sensitivity immunoassay) kits (Biosource International, Inc, Camarillo, California, USA). The instructions from the manufacturer of the kits were strictly followed. Plates were read at 450 nm using a Packard Spectracount® plate reader and I SMART 2.0 software. Standards as well as positive and negative controls were run with each plate. For the various cytokines measured, the minimal detectable concentrations were interleukin (IL)-18; 12.5 pg/mL, interferon (IFN)-γ; <4.0 pg/mL, IL-6; 2.0 pg/mL, IL-8; <5.0 pg/mL, IL-12; <2.0 pg/mL, IL-1ra; 4.0 pg/mL, IL-4; 2.0 pg/mL, IL-10; <1.0 pg/mL, soluble Tumor Necrosis Factor- receptor (sTNF-R1) p55; 50.0 pg/mL, sTNF-R11, p75; 100.0 pg/mL and Transforming Growth Factor (TGF-β; <15.6 pg/mL). To release TGF-β from latent complexes and to make it accessible for measurement, the serum sample was incubated with an extraction solution at room temperature for 10 minutes, followed by centrifugation.

Other assays

For light microscopy, biopsies from the noma lesions were taken from six, randomly selected patients. The children had no record of prior or current antibiotic therapy at the time of biopsy. The biopsies were placed in buffered formalin and tissue sections prepared by routine histological techniques. The tissues were stained with hematoxylin and eosin as well as with Brown-Brenn stain, which utilizes a modified Gram stain of crystal violet, Gram's iodine and basic fuchsin, and is a method for differential staining of Gram-positive and Gram-negative bacteria in tissue sections. The microscopic study was carried out for us in the Department of Oral Pathology at the University of Maryland Dental School (courtesy of Dr. Mark A. Reynolds).
Polymerase chain reaction methods were used to determine the presence of specific bacteria in oral samples collected from six, randomly selected noma cases. This was performed in the laboratory of Dr. J. Slots at the University of Southern California, Los Angeles, California. Details of the tissue sampling procedures have been published already [24].
Blood hemoglobin and plasma levels of vitamin A, vitamin C, zinc, albumin were also analyzed as previously described [9].

STATISTICAL ANALYSIS

The data are expressed as means ± standard deviation (SD). Statistical analyses were carried out using SPSS 11.5 for windows (SPSS Inc., Chicago, ILL). Differences were considered statistically significant when $p<0.05$. Cytokine levels in the various groups were in a skewed distribution. The values were therefore transformed logarithmically prior to analysis. Comparison of the serum cytokine levels between groups was by analysis of variance (ANOVA), with the Tukey-Kramer multiple comparison test. For the purposes of this study, the cytokines were classified as pro-inflammatory (IFN-γ, IL-6, IL-8, IL-18) and anti-inflammatory (IL-4, IL-10, TGF-β). Also studied as indices of their respective precursors (TNF-α and IL-1) were the TNF receptors (sTNFR-p55 and sTNFR-p75) and the interleukin-1 receptor antagonist, IL-1ra. The TNF-α and IL-1 have very short plasma half-lives and their concentrations are extremely low relative to the levels of their receptors [25]. It must be emphasized that many poorly understood factors affect blood cytokine concentrations [26], and that a given cytokine may behave as pro- or anti-inflammatory depending on its amount, nature of activating signals and other factors [20].
Blood hemoglobin and plasma retinol, total ascorbic acid, zinc, albumin levels in the neighborhood village children without noma and children with noma were normally distributed and therefore compared by the unpaired t test.

RESULTS

Figure 1 is a case of fresh noma in an underprivileged child with a fairly recent history of measles prior to the rapid development of the externally visible, destructive gangrene involving the right maxilla and mandible. The child presented with hair discoloration and edema of the lower limbs, both characteristic features of the kwashiorkor type of protein-energy malnutrition. The intra-oral destruction

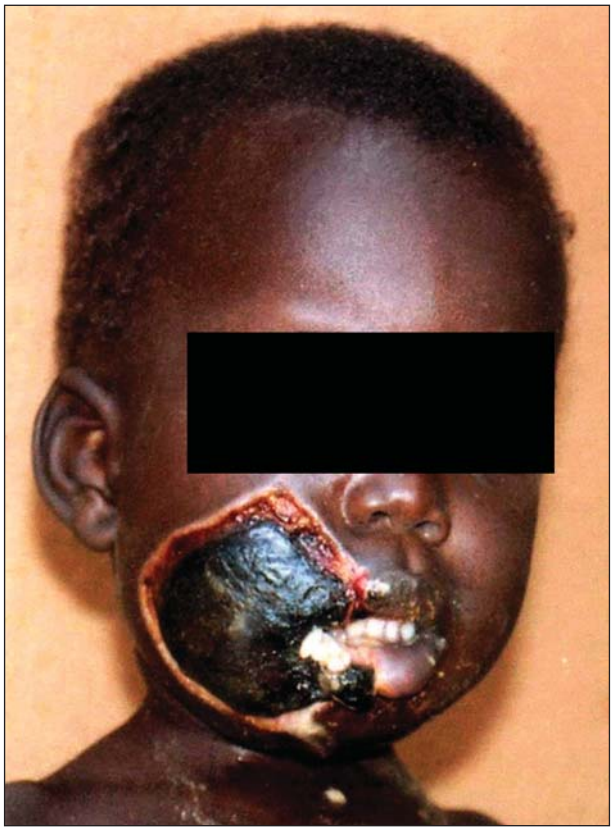

Figure 1
Fresh noma in a malnourished Nigerian child aged 2.5 yrs, with a prior history of measles and malaria. At the time of examination, the child weighed 8.0 kg and had a length of 74 cm.

Table 1
Plasma levels of nutrients and other factors in the study groups[#]

Item	Village control	Noma group
Vitamin A (μMol/L)	1.45 ± 0.68[ϕ] (n=32)	0.86 ± 0.51[ϕ] (n=28)
Vitamin C (μMol/L)	9.1 ± 4.4* (n=29)	6.73 ± 4.2* (n=35)
Zinc (μMol/L)	12.9 ± 2.8[ϕ] (n=28)	8.7 ± 1.6[ϕ] (n=26)
Albumin (g/L)	32.7 ± 3.2[ϕ] (n=21)	27.5 ± 3.8[ϕ] (n=16)
Hemoglobin (g/L)[‡]	86.3 ± 12.6[ϕ] (n=25)	67.4 ± 17.3[ϕ] (n=31)

[#] Data on urban control already reported [9]. Data are expressed as mean ± SD.
[‡] Blood hemoglobin analysis was carried out by our collaborating center in Nigeria.
* Significantly different (p<0.05).
[ϕ] Significantly different (p<0.001).

was more extensive than the externally visible lesion which had a clearly defined border. The two and a half-year old child was markedly growth-retarded with a length of 74 cm and body weight of 8.0 kg which were both lower than the reference values for a 2-years-old child [27].

Plasma levels of some antioxidant nutrients, albumin and blood hemoglobin concentration observed in the children with noma and their village counterparts without noma are shown in *table 1*. Noma produced significant reductions in plasma levels of vitamin A (p<0.001), vitamin C (p<0.05) and zinc (p<0.001) compared to the values in the neighborhood village children. It must be emphasized that plasma levels of these antioxidant micronutrients are not accurately reflective of nutritional status but can be due, in part, to infections [9, 28]. Similarly marked reductions (p<0.001) in albumin and blood hemoglobin were observed in children with noma compared to those without noma.

Histopathological study of the slides from biopsies revealed the following general observations. Necrosis was noticed in the epidermis with acanthosis around the rim of the epithelium. At the margins of the ulcer, there was hyperplasia and the epithelial cells displayed ballooning. In the dermis, there was a heavy infiltrate of primarily polymorphonuclear neutrophils (PMNs) and mononuclear cells. In one of the six samples, there was a predominance of plasma cells. Collagen bundles were separated possibly due to the presence of oedema. Blood vessels were dilated and their lumen filled with inflammatory cells. Vascular walls did not appear to be thickened. A polymicrobial layer was composed of small and large cocci (some diplococci and tetracocci), cocci arranged in chains and gram-negative cocci-bacilli. In that particular sample, PMNs and macrophages appeared to be full of phagocytosed bacteria.

Table 2 summarizes the serum levels (pg/mL) of the various cytokines in the three study groups. For each cytokine, there was marked individual variability among children in the same experimental group. Relative to the urban elite, control children, the children with noma showed significant (p<0.01 or <0.001) elevations in serum concentrations of IL-18 (+ 223%), IL-6 (+465%), IFN-γ (+186%), IL-8 (+2950%), IL-1ra (+37%), sTNFR-p55 (+109%), and sTNFR-p75 (+137%). The victims of noma also showed non-significant elevations in the levels of IL-4, IL-10 and TGF-β when compared to their urban control counterparts *(table 2)*. Similarly, the socioeconomically-deprived neighborhood village children potentially at risk of noma, showed significant changes in the serum levels of IL-18 (+139%), IL-6 (+570%), IL-12 (+101%), IFN-γ (+1328%), IL-8 (+6250%), IL-1ra (+22%), sTNFR-p55 (+11%), sTNFR-p75 (+99%), with hardly any differences in the anti-inflammatory cytokines except for IL-10 (+87%), when compared with urban control values. In comparison with the noma children, the socioeconomically-deprived neighborhood village children without noma, presented with significantly elevated serum levels of IL-12 (+103%), IFN-γ (+400%), IL-10

Table 2
Serum cytokine levels in children

Cytokine (pg/mL)	Urban control	Village control	Noma	P value
IL-18	459±291[a,c] (n=45)	1096±416[c] (n=28)	1482±1034[a] (n=26)	[a,c] p<0.001
IL-6	20±40[a,c] (n=45)	134±316[c] (n=50)	113±135[a] (n=27)	[a] p<0.01 ; [c] p<0.05
IL-12	176±104[c] (n=24)	354±230[b,c] (n=30)	174±146[b] (n=15)	[b,c] p<0.001
IFN-γ	0.7±1.0[c] (n=45)	10±4[b,c] (n=13)	2±4[b] (n=20)	[b,c] p<0.001
IL-8	2.0±2.4[a,c] (n=16)	127±239[c] (n=41)	61±141[a] (n=19)	[a] p<0.01; [c] p<0.05
IL-1ra	546±711[a,c] (n=24)	669±442[c] (n=30)	746±552[a] (n=26)	[a] p<0.01
sTNFR-p55	2460±1310[a] (n=23)	2730±970 (n=23)	5140±4390[a] (n=15)	[a] p<0.01
sTNFR-p75	7750±3590[a,c] (n=23)	15,420±8180[c] (n=25)	18,350±11000[a] (n=16)	[a,c] p<0.01
IL-10	6.1±15.4[c] (n=40)	11.4±14.3[c] (n=53)	7.9+6.2 (n=28)	[c] p<0.01
IL-4	2.5±0.7 (n=24)	3.0±1.4 (n=16)	3.0±1.0 (n=19)	
TGF-β	11,153±8467 (n=21)	16,971±16340 (n=13)	15,911±12999 (n=16)	

Data are expressed a means + SD.
Values with the same superscript for each cytokine are significantly different.

Table 3
Identification of bacteria in noma lesions

Microorganisms	Positives/number of patients sampled
Prevotella intermedia	5/6
Tannerella forsynthesis (formerly *Bacteroides forsythus*)	5/6
Porphyromonas gingivalis	3/6
Campylobacter rectus	3/6
Treponema denticola	3/6
Eikenella corrodens	2/6

(+44%) and non-significant reductions in concentrations of IL-18 (-26%), sTNFR-p55 (-47%), sTNFR-p75 (-16 %).

What was clear from the serum cytokines studied was that in both the noma victims and the underprivileged neighborhood village children without noma, the concentrations of the circulating pro-inflammatory cytokines (including the TNFR-p55, TNFR-p75 and IL-1ra) were much higher than in the urban control children, with the noma children more affected than their village counterparts. The increase in levels of the anti-inflammatory cytokines was less marked relative to the pro-inflammatory cytokines *(table 2)*. The marked variability in cytokine levels between individuals in the same group could result from baseline, pre-existing host factors including other subclinical infections, timing of sample collection relative to onset of infections, duration of malnutrition in the underprivileged groups, severity of sepsis and other conditions including the presence of genetic polymorphisms which might affect patients' responses to a stimulus [23, 29, 30].

The bacteria observed most commonly in the six samples subjected to analysis using the Polymerase Chain Reaction were *Prevotella intermedia* (83%), *Tannerella forsynthesis* (formerly *Bacteroides forsythus*) (83%), *Porphyromonas gingivalis* (50%), *Campylobacter rectus* (50%) and *Treponema denticola* (50%) *(table 3)*.

DISCUSSION

Many lesions/diseases, particularly in poor countries, are caused by complex synergistic interactions between parasites, fungi, viruses and bacteria with an underlying basis of chronic malnutrition, thus making it difficult to understand the role of specific pathogens. For example, malaria and intestinal parasitisms are quite common in our study sites [11, 31]. All the children examined in the present study were however devoid of other overt clinical infections. Ongoing studies in our laboratory indicate that malaria produces very profound increases in the production of the cytokines (particularly IL-6, TNF-α and the TNF receptors, etc.) compared with observations in our noma patients (Enwonwu *et al*., unpublished findings). This observation is consistent with recent findings in impoverished children with malaria in Mali, West Africa [32].

Noma is a bacterial disease as exemplified by the histopathological findings and clinical response to appropriate antibiotics [10, 22]. Nonetheless, the pathogenesis of noma is still poorly understood. The human oral cavity contains an estimated 300-500 bacterial species, and it is possible that the destruction typified by noma results from disorders of the local endogenous microflora and/or the presence of newcomer organisms. In such a situation, linkage between a specific microbe and the disease is not easy [33]. Noma is a polymicrobial infection and earlier studies in our laboratory demonstrated high recovery of *Fusobacterium necrophorum*, *Prevotella intermedia*, alpha-hemolytic streptococci and *Actinomyces* spp. from active sites of the lesion in Nigerian children [10, 32]. Arguments were presented in favour of *F. necrophorum* as a key trigger organism [10, 16]. The association and virulence of *F. necrophorum* with necrobacillosis in wallabies, and the similarity of this disease with noma in humans resulted in the proposal that this microorganism may be involved in the etiology of human noma [7]. Virulence factors of *F. necrophorum* include a classic endotoxin, a dermo-necrotic toxin, and other toxins and enzymes which will lead to tissue destruction and the production of a low oxidation-reduction potential [1]. Advanced noma is an open lesion which can be easily contaminated, and a more recent, culture-independent molecular study has highlighted the complex diversity of microorganisms associated with the disease [17]. In the present study *(table 3)*, *Tannerella forsythensis* (formerly *Bacteroides forsythus*), *Porphyromonas gingivalis*, *Camplyobacter rectus* and *Treponema denticola* were added to the list of organisms associated with the lesion. These bacteria are among those most often cultivated at high levels from chronic periodontitis in adults and they release enzymes such as collagenase, trypsin-like enzyme, phospholipase A, neuraminidase, arylsulfatase, and fibronectin-degrading enzyme which are capable of degrading host tissues [34]. Additionally, oral epithelial cells in contact with these pathogens secrete a variety of inflammatory mediators, which, among many other biological actions, promote neutrophil infiltration, activation of matrix metalloproteinases, and impaired regulation of skeletal homeostasis [35-38].

Noma is believed to start as a periodontal lesion/disease [1], and the general concept is that no more than 20% of the variability in periodontal disease (PD) expression can be accounted for by the presence or absence of bacterial plaque, thus suggesting a significant contribution of the host responses to disease expression [39, 40]. Many studies have emphasized the astonishingly rapid, extensive necrosis and destruction of the oro-facial tissues in acute noma, as well as the rarity of secondary hemorrhage, despite the proximity of major arterial vessels in the areas affected [3-6]. The pattern of development of noma lesion thus suggests an immunopathological reaction to oral microbial organisms. Cytokines are important in the immunopathology of many diseases [36, 41]. The potential relevance of our findings *(table 2)* to the pathogenesis of noma relate to the broad classification of oral mucosal inflammations into those due to excessive production of a key Th1 or Th2 effector cytokine and/or to diminished production of a regulatory cytokine, with the suppressor cytokines such as TGF-β and IL-10 included in the latter [42]. Cytokines are produced by immune and non-immune cells, and it is now clear that in response to bacterial products, oral epithelial cells, sub-epithelial cells and other resident cells secrete several pro-inflammatory cytokines and chemokines such as IL-1β, IL-6, IL-8, IL-18, PGE2 and TNF-α, among others [34, 35, 38, 43]. The pro-inflammatory cytokines TNF-α and IL-18 not only stimu-

late expression of the matrix metalloproteinases [44] but also, along with IL-6, play a role in pathological bone loss [45].

Our studies *(table 2)* demonstrated very statistically significant elevations in levels of the pro-inflammatory cytokines (including the soluble cytokine inhibitors sTNFR and IL-1ra) in noma children and neighborhood village subjects compared to the elite urban children. There were less marked increases in the serum levels of the anti-inflammatory cytokines *(table 2)*. These findings were consistent with reported observations in various conditions of sepsis [23, 46]. The high levels of many pro- and anti-inflammatory cytokines in the neighborhood village children relative to the urban group were a reflection of the fact that children in the former group were generally not healthy. Multiple infections are the rule rather than the exception in poor, African, rural communities, and we could not completely rule out the presence of some other subclinical infections in both our neighborhood village groups and the noma victims. We had earlier reported very high prevalence of growth retardation, an index of widespread malnutrition and poor health status in apparently normal Nigerian village children [9, 28, 47]. There is evidence that malnutrition as exemplified by stunting and weight loss is more severe in children with noma than in their village neighborhood counterparts without the disease [45]. This was also confirmed in the present study *(table 1)*. These pre-existing conditions would affect serum cytokine levels as reflected in malnourished African children without overt infections [18, 26]. There were no statistically significant differences in cytokine levels observed in this study between the noma victims and their neighborhood village counterparts. Relative to the neighborhood village children, the noma group demonstrated increases in the serum levels of sTNFR-p55 (+88%), sTNFR-p75 (+19%) and IL-18 (+35%) *(table 2)*. These differences, although statistically nonsignificant, might have some practical clinical importance and therefore will be subjected to further study in a larger group of noma patients. The multifunctional cytokine, IL-18, which augments both innate and acquired immunity, is expressed by various types of immune and non-immune cells, epithelial cells, osteoblasts, adrenal cortex, among others [48]. Neutrophil proteinase 3 (PR3)-mediated induction of bioactive IL-18 secretion from oral epithelial cells *via* a caspase-independent pathway has been reported [35]. It induces gene expression and synthesis of TNF, IL-1, Fas ligand and several chemokines, activates MMP, and is implicated in several mucosal immune disorders [49-51]. Increased blood levels of IL-18 have been reported in sepsis [52], ulcerative diseases [53], measles [54], and malaria (*Plasmodium falciparum*) [55], among other diseases. There are suggestions of an association between the severity of outcome of sepsis and the level of circulating IL-18 [56]. The significantly increased levels of IL-6 in the neighborhood village and noma children (*table 2*) would promote hepatic acute-phase protein response [57], which is a common finding in infected, protein-energy-deficient children [18]. The serum levels of IL-6, usually observed in the village children with uncomplicated malaria, was about 3 to 4 fold the levels reported in the present study, thus ruling out malaria as a major confounding factor in this study (Enwonwu *et al.*, unpublished observation).

The cytokine changes seen in noma patients and in deprived neighborhood village children at risk of noma *(table 2)* showed close similarities to reported findings in other diseases characterized by necrotic lesions. In Crohn's disease (CD), an inflammatory bowel disease reportedly caused by immunological overreaction to commensal gut bacteria, mucosal and systemic concentrations of many pro- and anti-inflammatory cytokines, particularly TNF and IL-18 are elevated [36], and there is evidence that blockade of IL-18 ameliorates the murine model of CD [53]. Necrotizing enterocolitis (NEC) affects predominantly low birth weight/premature infants and shows elevated levels of various pro-inflammatory cytokines [58]. Similarly, studies have confirmed that the increased intestinal permeability and mucosal injury in impoverished, malnourished Gambian children is due to TNF-α-mediated matrix degradation [14]. The complexity of changes in serum cytokine levels observed in noma patients and the village children resembles alterations reported in severe sepsis by others [19]. Studies are needed to examine the relationship between serum levels of these cytokines and their expression/concentration at the tissue sites of production in both noma victims, and their socioeconomically-underprivileged neighborhood counterparts potentially at risk of noma. Additionally, we would investigate the potential use of oral mucosal cytokine expression as early markers of malnourished, infected children at risk of noma, while we continue to search for the unknown factor that elicits the lesion, in some malnourished children and not in others residing in the same deprived communities.

Acknowledgements. *USPHS Grant DE43TW00907A from the US National Institutes of Health (Fogarty International Center and the NIDCR) and a Grant from Nestle Foundation, Lausanne, Switzerland supported this project.*

REFERENCES

1. Enwonwu CO, Falkler WA, Idigbe EO. Oro-facial gangrene (noma/cancrum oris): pathogenetic mechanisms. *Crit Rev Oral Biol Med* 2000; 11: 159.
2. Baratti-Mayer D, Pittet B, Montandon D, Bolivar I, Bornand JE, Hugonnet S, Jaquinet A, Schrenzel J, Pittet D, Geneva Study Group on Noma. Noma: an ″infectious″ disease of unknown aetiology. *Lancet Infec Dis* 2003; 3: 419.
3. Tempest MN. Cancrum oris. *Br J Surg* 1966; 53: 949.
4. Enwonwu CO. Epidemiological and biochemical studies of necrotizing ulcerative gingivitis and noma (cancrum oris) in Nigerian children. *Arch Oral Biol* 1972; 17: 1357.
5. Jelliffe DB. Infective gangrene of the mouth (cancrum oris). *Pediatrics* 1952; 9: 544.
6. Evrard L, Laroque G, Glineur R, Daelemans P. Noma: clinical and evolutive aspect. *Acta Stomatol Belg* 1996; 93: 17.
7. Enwonwu CO. Noma: a neglected scourge of children in sub-Saharan Africa. *Bulletin World Hlth Organ* 1995; 73: 541.
8. Marck KW. A history of noma, the ″face of poverty″. *Plast Reconstr Surg* 2003; 111: 1702.
9. Enwonwu CO, Falkler Jr. WA, Idigbe EO, Afolabi BM, Ibrahim M, Onwujekwe D, Savage O, Meeks VI. Pathogenesis of cancrum oris (noma): confounding interactions of malnutrition with infection. *Am J Trop Med Hyg* 1999; 60: 223.

10. Falkler Jr. WA, Enwonwu CO, Idigbe EO. Isolation of Fusobacterium necrophorum from cancrum oris (noma). *Am J Trop Med Hyg* 1999; 60: 150.

11. Idigbe EO, Enwonwu CO, Falkler WA. Living conditions of children at risk for noma: Nigerian experience. *Oral Dis* 1999; 5: 156.

12. Solomons NW, Mazariegos M, Brown KH, Klasing K. The underprivileged, developing country child: environmental contamination and growth failure revisited. *Nutr Rev* 1993; 51: 327.

13. Lunn PG. Growth retardation and stunting of children in developing countries. *Br J Nutr* 2002; 88: 109.

14. Campbell DI, Murch SH, Elia M, Sullivan PB, Sanyang MS, Jobarteh B, Lunn PG. Chronic T cell-mediated enteropathy in rural West African children: relationship with nutritional status and small bowel function. *Pediatr Res* 2003; 54: 306.

15. Solomons NW. Environmental contamination and chronic inflammation influence human growth potential. *J Nutr* 2003; 133: 1237.

16. Falkler WA, Enwonwu CO, Ewell AJ, Idigbe EO. Isolation of fusobacteria from the oral cavities of malnourished Nigerian children living in agricultural and herding villages. *Oral Dis* 2000; 6: 103.

17. Paster BJ, Falkler Jr. WA, Enwonwu CO, Idigbe EO, Savage KO, Levanos VA, Tamer MA, Ericson RL, Lau CN, Dewhirst FE. Prevalent bacterial species and novel phylotypes in advanced noma lesions. *J Clin Micro* 2002; 40: 2187.

18. Sauerwein RW, Mulder JA, Mulder L, Lowe B, Peshu N, Demacker PN, van der Meer JW, Marsh K. Inflammatory mediators in children with protein-energy malnutrition. *Am J Clin Nutr* 1997; 65: 1534.

19. Gogos CA, Drosou E, Bassaris HP, Skoutelis A. Pro- *versus* Anti-inflammatory cytokine profile in patients with severe sepsis: a marker for prognosis and future therapeutic options. *J Infect Dis* 2000; 181: 176.

20. Cavaillon JM. Pro- *versus* anti-inflammatory cytokines: myth or reality. *Cell Mol Biol* 2001; 47: 695.

21. Rehan NE, Tafida DS. Low birth weight in Hausa infants. *Niger J Paediatr* 1981; 8: 35.

22. Falkler Jr. WA, Enwonwu CO, Idigbe EO. Microbiological understandings and mysteries of noma (cancrum oris). *Oral Dis* 1999; 5: 150.

23. Enwonwu CO. Influence of socio-economic conditions on dental development in Nigerian children. *Arch Oral Biol* 1973; 18: 95.

24. Contreras A, Falkler Jr. WA, Enwonwu CO, Idigbe EO, Savage KO, Afolabi MB, Onwujekwe D, Rams TE, Slots J. Human herpesviridae in acute necrotizing ulcerative gingivitis in children in Nigeria. *Oral Microbiol Immunol* 1997; 12: 259.

25. Rivera S, Sattler FR, Boyd H, Auffenberg T, Nakao S, Moldawer LL. Urinary cytokines for assessing inflammation in HIV-associated wasting. *Cytokine* 2001; 13: 305.

26. Bone RC. Toward a theory regarding the pathogenesis of the systemic inflammatory response syndrome: what we do and do not know about cytokine regulation. *Crit Care Med* 1996; 24: 163.

27. NCHS. *Growth Curves for Children from Birth to 18 years*. Washington, DC, United States: Department of Health, Education and Welfare. DHEW Publications, PHS, 1977; 78-1650.

28. Phillips RS, Enwonwu CO, Okolo S, Hassan A. Metabolic effects of acute measles in chronically malnourished Nigerian children. *J Nutr Biochem* 2004; 15(5): 281.

29. Bienvenu JA, Monneret G, Gutowski MC, Fabien N. Cytokine assays in human sera and tissues. *Toxicology* 1998; 129: 55.

30. Jason J, Archibald LK, Nwanyanwu OC, Byrd MG, Kazembe PN, Dobbie H, Jarvis WR. Comparison of serum and cell-specific cytokines in humans. *Clin Diagn Lab Immunol* 2001; 8: 1097.

31. Enwonwu CO, Afolabi BM, Salako LO, Idigbe EO, Bashirelahi N. Increased plasma levels of histidine and histamine in falciparum malaria: relevance to severity of infection. *J Neural Transm* 2000; 107(11): 1273.

32. Lyke KE, Burges R, Cissoko Y, Sangare L, Dao M, Diarra I, Kone A, Harley R, Plowe CV, Doumbo OK, Sztein MB. Serum levels of the proinflammatory cytokines interleukin-1 beta (IL-1beta), IL-6, IL-8, IL-10, tumor necrosis factor alpha, and IL-12(p70) in Malian children with severe Plasmodium falciparum malaria and matched uncomplicated malaria or healthy controls. *Infect Immun* 2004; 72(10): 5630-7.

33. Relman DA. The search for unrecognized pathogens. *Science* 1999; 284: 1308.

34. Haake SK, Nisengard RJ, Newman MG, *et al.* Microbial interactions with host in periodontal disease. In: Newman MG, Takei HH, Carranza FA, eds. *Carranza's clinical periodontology, 9th edition*. Philadelphia: W.B. Saunders Co, 2002: 132.

35. Sandros J, Karlsson C, Lappin DF, Madianos PN, Kinane DF, Papapanou PN. Cytokine responses of oral epithelial cells to Porphyromonas gingivalis infection. *J Dent Res* 2000; 79: 1808.

36. Brandtzaeg P. Inflammatory bowel disease: clinics and pathology. Do inflammatory bowel disease and periodontal disease have similar immunopathogenesis? *Acta Odontol Scand* 2001; 59: 235.

37. Sugawara S, Uehara A, Nochi T, Yamaguchi T, Ueda H, Sugiyama A, Hanzawa K, Kumagai K, Okamura H, Takada H. Neutrophil proteinase 3-mediated induction of bioactive IL-18 secretion by human oral epithelial cells. *J Immunol* 2001; 167: 6568.

38. Rouabhia M, Ross G, Page N, Chakir J. Interleukin-18 and gamma interferon production by oral epithelial cells in response to exposure to Candida albicans or lipopolysaccharide stimulation. *Infect Immun* 2002; 70: 7073.

39. Kornman KS, Loe H. The role of local factors in the etiology of periodontal diseases. *Periodontology* 2000; 2: 83.

40. Dayan S, Stashenko P, Neiderman R, Kupper TS. Oral epithelial over expression of IL-1α causes periodontal disease. *J Dent Res* 2004; 83: 786.

41. Seymour GJ, Gemmell E. Cytokines in periodontal disease: where to from here? *Acta Odontol Scand* 2001; 59: 167.

42. Strober W, Fuss I, Kitani A. Regulation of experimental mucosal inflammation. *Acta Odontol Scand* 2001; 59: 244.

43. Tervahartiala T, Koski H, Xu JW, Hayrinen-Immonen R, Hietanen J, Sorsa T, Konttinen YT. Tumor necrosis factor-alpha and its receptors, p55 and p75, in gingiva of adult periodontitis. *J Dent Res* 2001; 80: 1535.

44. Birkedal-Hansen H. Role of cytokines and inflammatory mediators in tissue destruction. *J Periodontol Res* 1993; 28: 500.

45. Skerry TM. The effects of the inflammatory response on bone growth. *Eur J Clin Nutr* 1994; 48(Suppl): S190.

46. Hack CE, Aarden LA, Thus LG. Role of cytokines in sepsis. *Adv Immunol* 1997; 66: 101.

47. Enwonwu CO, Phillips RS, Ferrell CD. Temporal relationship between the occurrence of fresh noma and the timing of linear growth retardation in Nigerian children. *Trop Med Intern Health* 2005; 10: 65.

48. Okamura H, Tsutsui H, Kashiwamura SI. Interleukin-18: a novel cytokine that augments both innate and acquired immunity. *Adv Immunol* 1998; 70: 281.

49. Biet F, Locht C, Kremer L. Immunoregulatory functions of interleukin 18 and its role in defense against bacterial pathogens. *J Mol Med* 2002; 80: 147.

50. Kashiwamura SI, Ueda H, Okamura H. Roles of interleukin-18 in tissue destruction and compensatory reactions. *J Immunother* 2002; 25(Suppl 1): S4.

51. Eaton AD, Xu D, Garside P. Administration of exogenous interleukin-18 and interleukin-12 prevents the induction of oral tolerance. *Immunology* 2003; 108: 196.

52. Oberholzer A, Feilner A, Hentze H. Sepsis after severe injury interrupts caspase-dependent processing of interleukin-18. *J Trauma* 2000; 49: 11.

53. Ishikura T, Kanai T, Uraushihara K, Iiyama R, Makita S, Totsuka T, Yamazaki M, Sawada T, Nakamura T, Miyata T, Kitahora T, Hibi T, Hoshino T, Watanabe M. Interleukin-18 overproduction exacerbates the development of colitis with markedly infiltrated macrophages in interleukin-18 transgenic mice. *J Gastroenterol Hepatol* 2003; 18: 960.

54. Okada H, Sato TA, Katayama A. Comparative analysis of host responses related to immunosuppression between measles patients and vaccine recipients with live attenuated measles vaccines. *Arch Virol* 2001; 146: 859.

55. Nagamine Y, Hayano M, Kashiwamura SI. Involvement of interleukin-18 in severe Plasmodium falciparum malaria. *Trans R Soc Trop Med Hyg* 2003; 97: 236.

56. Oberholzer A, Steckholzer U, Kurimoto M. Interleukin-18 plasma levels are increased in patients with sepsis compared to severely injured patients. *Shock* 2001; 16: 411.

57. Gabay C, Kushner I. Acute-Phase proteins and other systemic responses to inflammation. *N Engl J Med* 1999; 340: 448.

58. Pender SL, Braegger C, Günther U, Monteleone G, Meuli M, Schuppan D, Macdonald TT. Matrix metalloproteinases in necrotizing enterocolitis. *Pediatr Res* 2003; 54: 160.