Influence of Host Factors on Immunoglobulin G Concentration in Oral Fluid Specimens

Timothy C. Granade,1* Susan K. Phillips,1 Wendy Kitson-Piggott,2 Perry Gomez,3 Bisram Mahabir,4 Herbert Oleander,3 J. Richard George,1† James Baggs,1‡ and Bharat Parekh1

Division of AIDS, STD, and TB Laboratory Research, National Center for Infectious Diseases, Centers for Disease Control and Prevention, Atlanta, Georgia 30333; Caribbean Epidemiology Centre2 and Ministry of Health, Trinidad and Tobago,3 Port-of-Spain, Trinidad, British West Indies; and Ministry of Health and Environment, Nassau, Bahamas3

Received 31 July 2001/Returned for modification 5 September 2001/Accepted 22 October 2001

The influence of host factors (tobacco use, dentition, bleeding gums, oral rinsing, nasal medications, and time since the last meal) on immunoglobulin G (IgG) concentration in oral fluids (OF) was determined by univariate and multivariate analysis. Significant differences in IgG concentration were found to be associated with human immunodeficiency virus (HIV) status (HIV antibody positive, +16.60 μg/ml, P = 0.0001), sex (female, +1.23 μg/ml, P = 0.004), dentition (+2.83 μg/ml, edentulous versus dentulous, P = 0.0001), bleeding gums (+6.35 μg/ml, P = 0.0001), and time since the last meal (+3.55 μg/ml, >6 h, P = 0.0001). These factors could impact diagnostic methods that rely on the immunoglobulin concentration in OF specimens.

Recently, specialized devices have been developed to standardize the collection of oral fluids (OF) for use in detecting antibodies to infectious agents including human immunodeficiency virus (HIV) types 1 and 2 and hepatitis, dengue, measles, mumps, and rubella viruses (4, 5, 13, 16, 17, 21). OF is a complex mixture of secretions, transudates, metabolic by-products, and ingested materials (19). The immunological reactivity in OF with antigens from HIV has been shown to be from plasma-derived immunoglobulin G (IgG) that reaches the oral cavity by saliva flow (21). However, many factors could impact diagnostic methods that rely on the immunoglobulin concentration in OF specimens.

Statistical analysis of the data set was performed to establish the mean, median, and distribution characteristics of the IgG concentrations determined. The data set was further analyzed by univariate and multivariate analyses. Significant differences in IgG concentration were found to be associated with human immunodeficiency virus (HIV) status (HIV antibody positive, +16.60 μg/ml, P = 0.0001), sex (female, +1.23 μg/ml, P = 0.004), dentition (+2.83 μg/ml, edentulous versus dentulous, P = 0.0001), bleeding gums (+6.35 μg/ml, P = 0.0001), and time since the last meal (+3.55 μg/ml, >6 h, P = 0.0001). These factors could impact diagnostic methods that rely on the immunoglobulin concentration in OF specimens.

Blood was collected by venipuncture, and serum was used for the reference HIV antibody testing. The OF was collected in duplicate as previously described using the U.S. Food and Drug Administration-licensed Orasure device (7). The specimens were processed according to the manufacturer’s instructions, and the duplicate specimens were pooled and aliquoted for immediate use and for storage at −20°C. The quantitation of IgG in the OF specimens was performed using an in-house EIA (7).

The ratio of men to women was 2:1, and the age distribution ranged from less than 10 to greater than 65 years, with 70% of the population in the 19- to 39-year-old age group. The time since the last meal reflected the time of day that the specimens were collected, with 19.1% reported more than 10 h since the last meal, whereas most participants (52.3%) provided specimens within 1 to 3 h of eating. Smoking was reported by 29.7% of the respondents, while only a few individuals (0.5%) indicated chewing tobacco (Table 1). Mouth washing or oral rinsing was performed by 6.0% of individuals prior to coming to the col-
Only 142 individuals (3.2%) reported taking medications, such as antihistamines or secretory inhibitors, at the time of sample collection. The use of medications, including gums, all of their natural teeth, and 15.7% reported having bleeding gums at the time of sample collection. The use of medications, such as antihistamines or secretory inhibitors, was reported by only 142 individuals (3.2%).

The mean IgG concentration in OF was 17.1 µg/ml (standard deviation = 13.03 µg/ml), with a range of 0.0 µg/ml (undetectable in the OF sample in the IgG quantitative assay) to 100 µg/ml (upper limit of the IgG assay). The distribution of the IgG concentrations in the population was not normal but was modal and skewed to the right (data not shown).

The IgG concentrations in the OF specimens were analyzed according to the demographic and external factors using standard t tests (Fig. 1). The mean IgG concentration in OF of HIV-positive individuals (mean = 32.60 µg/ml, median = 26.92 µg/ml) was more than twice that of HIV-negative individuals (mean = 15.30 µg/ml, median = 13.00 µg/ml) (P = 0.0001). A significant difference of >1 µg/ml was also noted between males and females (17.60 versus 17.93 µg/ml, respectively; P = 0.004). No significant differences were observed related to smoking, chewing tobacco, or oral rinsing. Dentate individuals had an average OF IgG concentration of 15.99 µg/ml and edentate or partially dentate individuals averaged 18.82 µg/ml (P = 0.0001). The interval between eating and OF specimen collection was also correlated with increases in IgG concentration in OF specimens over time. The average concentration of IgG in OF specimens collected within 2 h of the last meal was 16.01 µg/ml. IgG concentrations steadily increased over time to 16.54 µg/ml at 2 to 3 h, 16.85 µg/ml at 3 to 6 h, and 19.56 µg/ml for time periods >6 h since the last meal (P = 0.0001). The average OF IgG concentration was higher in individuals ≥50 years of age than in younger individuals (20.44 versus 16.90 µg/ml, respectively; P = 0.0019) (n = 243).

Multivariate analysis was performed to determine the interaction of demographic and external factors with IgG concentration in OF specimens using a multiple linear regression model (Table 2). HIV status, sex, age, the presence of natural teeth, the presence of bleeding gums, cigarette smoking, the use of chewing tobacco, and the number of hours since the last meal were entered into the model. A large increase in the mean OF IgG concentration (+13.82 µg/ml, P = 0.0001) was observed in the HIV-positive individuals compared to the population arithmetic mean of 17.10 µg/ml. Persons reporting bleeding gums also had a significant increase in OF IgG concentration (+4.98 µg/ml, P = 0.0001). Although, in univariate analysis, a difference in IgG concentration was associated with sex, no such difference was observed in the multivariate analysis. Increases in OF IgG concentration according to increasing age were also not observed. The use of an oral rinse and cigarette smoking were both associated with small decreases in OF IgG concentration, but only smoking was significant (−2.15 µg/ml, P = 0.0001). The lack of natural teeth was related to a significant increase in IgG (+1.19 µg/ml, P = 0.0001). As with the univariate analysis, OF IgG concentration significantly increased with increasing time since the last meal in the multivariate analysis (+2.41 µg/ml, P = 0.0001).

These data represent a large sample set for analysis of parameters that affect IgG concentration in Orasure specimens. Infection with HIV has been shown to induce a hypergamma-globulinemic state with serum antibody levels significantly increased, especially for the IgG class (20). Lu et al. noted this increase in OF IgG concentration in HIV-infected persons and postulated that the additional IgG was due to locally produced mucosal antibody and was based on increases in specific antibodies to HIV antigens (14). However, relative increases in IgG and HIV-specific IgG in OF are reflected in the parallel IgG increases in serum. These increases more likely represent a proportionate transudation rather than local synthesis.

Little information exists about the impact of tobacco products on the immune composition of OF. Cigarette smoking has been shown previously to cause significant changes in the immune system both systemically and on the mucosal surfaces (6, 11, 15). Previous studies have indicated a reduction of IgG concentration in serum among individuals who smoke (11, 22). Our data indicated no differences in IgG concentration in OF caused by smoking by univariate analysis but did show significant reductions in OF IgG concentration using the multivariate model when the data were stratified according to HIV status. The effect of chewing tobacco products could increase the IgG antibody concentrations due to the increased salivary flow that would wash the IgG from the gingival crevices, or the antibody concentration could be reduced as the antibodies are washed out and expeptorated or swallowed. A trend toward increased IgG concentration associated with chewing tobacco was indicated by both univariate and multivariate analysis, although neither was significant.

The effect of dentition on the concentration of immuno-

### Table 1. Numbers and percentages of study participants reporting or not reporting specific behaviors or conditions

| Characteristic                  | Yes (%)  | No (%)  | Not reported (%) |
|--------------------------------|----------|---------|------------------|
| Cigarette smoking              | 1,315(29.7) | 3,086(69.5) | 34(0.8)          |
| Chewing tobacco                | 24(0.5)   | 4,376(98.6) | 38(0.9)          |
| Oral rinsing                   | 266(6.0)  | 4,124(92.9) | 48(1.1)          |
| Natural teeth                  | 2,638(59.4) | 1,755(39.5) | 45(1.1)          |
| Bleeding gums                  | 698(15.7)  | 3,704(83.5) | 36(0.8)          |
| Use of cold or allergy         | 142(3.2)  | 4,248(95.8) | 45(1.1)          |

### Table 2. Effects of demographic and external factors on the concentration of IgG in Orasure OF specimens—multiple linear regression model

| Variable                      | Deviation from arithmetic mean (17.10) | P value |
|-------------------------------|----------------------------------------|---------|
| HIV positive                  | 13.82                                  | 0.0001  |
| Sex—male                     | −0.17                                  | 0.6611  |
| Cigarette smoking—yes        | −2.17                                  | 0.0001  |
| Chewing tobacco—yes          | 1.89                                   | 0.4274  |
| Mouth rinse—yes              | −0.91                                  | 0.2199  |
| Natural teeth—no             | 1.19                                   | 0.0019  |
| Bleeding gums—yes            | 4.98                                   | 0.0001  |
| Time (h) since last meal     |                                        |         |
| >2–3                         | 0.65                                   | 0.1815  |
| >3–6                         | 0.79                                   | 0.1047  |
| >6                           | 2.41                                   | 0.0001  |

* n = 4,448.
globulins in the oral cavity has been shown previously to have a mixed impact on immunoglobulin levels (1, 8, 9). Our data indicated a significant increase in IgG concentration in edentulous or partially dentate individuals. This rise in IgG concentration is likely due to the increased surface area available for transudation from the capillaries to the oral cavity, but the actual cause has not been delineated. Inflammation around the gum line and the gingival crevices has also been shown to increase gingival flow and thus to increase the transudation of serum components (22). If the inflammation is sufficient to cause actual bleeding into the oral cavity, all blood components including immunoglobulins would be expected to increase, and our data confirmed a significant increase in IgG concentration in OF of approximately 5 μg/ml in individuals with bleeding gums.

The use of mouthwashes and oral rinsing would be expected to decrease the IgG concentration in OF. However, multiple sequential samplings of OF using collection devices have shown no significant reductions in the amount of IgG in the sample (R. L. Zimmerman, L. F. Hofman, S. M. Adair, L. Lugalia, and K. D. McMahan, presented at the VIth International Conference on AIDS, San Francisco, Calif., 20 to 23 June 1990). Decreases in OF IgG concentration were observed for individuals who performed oral rinses, but they were not significant. Food ingestion would also be expected to reduce the amount of IgG in OF by the absorption of IgG into food.
and by swallowing. A decrease in OF IgG concentrations among participants in our study was observed in samples taken shortly after eating. The flow of IgG back into OF appeared to steadily increase after the meal, reaching the arithmetic mean IgG concentration in 2 to 3 h and rising to 2.40 μg/ml over the mean at >6 h concentration after eating.

These data indicate that the IgG antibody levels in Orasure OF specimens may be altered due to cigarette smoking, oral rinsing, tobacco chewing, individual dentition, bleeding gums, or food ingestion. Population demographic factors such as age and sex did not significantly change the IgG concentrations in OF or affect HIV antibody detection. These data are consistent with similar analyses that have examined the specific impact of such factors on HIV EIA performance (1, 3). However, these IgG differences may have an impact on the detection of antibodies to other infectious agents that generate a less vigorous immune response.

We thank Rosemae Bain, Candace Bain, and Ann Rolle (Bahamas) and Michelle Noriega and Gloria Chan (Trinidad and Tobago) for study coordination and technical assistance. We extend special thanks to Steve Soroka for additional statistical analysis and to Harrison Stetter for assistance with demographic data collection.

REFERENCES

1. Bagg, J., K. R. Perry, J. V. Parry, P. P. Mortimer, and T. J. Peters. 1991. The influence of dental status on the detection of IgG class anti-viral antibodies in human saliva. Arch. Oral Biol. 32:221–226.
2. Callacombe, S. J., R. S. Percival, and P. D. Marsh. 1995. Age-related changes in immunoglobulin isotypes in whole and parotid saliva and serum in healthy individuals. Oral Microbiol. Immunol. 10:202–207.
3. Emmons, W. W., S. R. Paparell, C. R. Decker, J. M. Shefield, and F. H. Lowe-Bey. 1995. A modified ELISA and Western blot accurately determine anti-human immunodeficiency virus type 1 antibodies in oral fluids obtained with a special collection device. J. Infect. Dis. 171:1406–1410.
4. Freirichs, R. R., N. Silaru, N. Eskes, P. Pagcharoenpol, A. Rodklai, S. Thangsupchaisri, and C. Woringa. 1994. Saliva-based HIV antibody testing in Thailand. AIDS 8:555–594.
5. Gallo, D., J. R. George, J. H. Fitchen, A. S. Goldstein, M. S. Hindahl, and the OraSure HIV Clinical Trials Group. 1997. Evaluation of a system using oral mucosal transudate for HIV-1 antibody screening and confirmatory testing. JAMA 277:254–258.
6. Gerrard, J. W., D. C. Heiner, C. G. Ko, J. Mink, A. Meyers, and J. A. Dosman. 1980. Immunoglobulin levels in smokers and non-smokers. Ann. Allergy 44:261–262.
7. Granade, T. C., S. K. Phillips, B. Parekh, P. Gomez, W. Kitson-Piggott, H. Oleander, B. Mahibir, W. Charles, and S. Lee-Thomas. 1998. Detection of antibodies to human immunodeficiency virus type 1 in oral fluids: a large-scale evaluation of immunoassay performance. Clin. Diagn. Lab. Immunol. 5:171–175.
8. Gronblad, E. A. 1982. Concentration of immunoglobulins in human whole saliva: effect of physiological stimulation. Acta Odontol. Scand. 40:87–95.
9. Gronblad, E. A., and K. Lindholm. 1987. Salivary immunoglobulin concentra-

Vol. 9, 2002  NOTES 197 tion in predentate and edentulous mouths. Scand. J. Dent. Res. 95:27–31.
10. Grundbacher, F. J. 1988. Variation in levels of immunoglobulins A, G, and M in human saliva. Arch. Oral Biol. 33:121–126.
11. Gulsvik, A., and M. K. Fagerhol. 1979. Smoking and immunoglobulin levels.
Lancet 84:49.
12. Hodinka, R. L., T. Nagashumugam, and D. Malamud. 1998. Detection of human immunodeficiency virus antibodies in oral fluids. Clin. Diagn. Lab. Immunol. 5:419–426.
13. Holm-Hansen, C., N. T. Constantine, and G. Haukenes. 1993. Detection of antibodies to HIV in homogenous sets of plasma, urine, and oral mucosal transudate samples using rapid assays in Tanzania. Clin. Diagn. Virol. 1:207–214.
14. Lu, X. S., J. F. Delfraissy, L. Grangeot-Keros, M. T. Rannou, and J. Pilott. 1994. Rapid and constant detection of HIV antibody response in saliva of HIV-infected patients: selective distribution of anti-HIV activity in the IgG isotype. Res. Virol. 145:369–377.
15. McMillian, S. A., J. P. Douglas, G. P. R. Archibald, E. E. McCrum, and A. E. Evans. 1997. Effect of low to moderate levels of smoking on serum immuno-
globulin concentrations. J. Clin. Pathol. 50:819–822.
16. Parry, J. V., K. R. Perry, and P. P. Mortimer. 1987. Sensitive assays for viral antibodies in saliva: an alternate to tests on serum. Lancet 1:72–75.
17. Perry, K. R., D. W. G. Brown, J. V. Parry, S. Panday, C. Pipkin, and A. Richards. 1993. Detection of measles, mumps, and rubella antibodies in saliva using antibody capture radioimmunoassay. J. Med. Virol. 40:235–240.
18. Raux, M., L. Finkelshtein, D. Salmon-Ceron, H. Bouchez, J. L. Excler, E. Dusouset, J. M. Grouin, D. Sicard, and C. Blondeau. 1999. Comparison of the distribution of IgG and IgA antibodies in serum and various mucosal fluids of HIV type 1-infected subjects. AIDS Res. Hum. Retrovir. 15:1365–1376.
19. Roitt, I., and T. Lehner. 1983. Oral immunity, p. 279–304. In Immunology of oral diseases, 2nd ed. Blackwell Publications, Oxford, England.
20. Scadden, D. T., and D. W. Golde. 1996. Growth factors in the treatment of HIV disease, p. 525–534. In S. Gupta (ed.), Immunology of HIV infection. Plenum Publishing Corporation, New York, N.Y.
21. Soto-Ramirez, L. E., I. Hernandez-Gomez, J. Sifuentes-Osornio, G. Barriga-Angulo, D. Duarte de Lima, M. Lopez-Porillo, and G. M. Ruiz-Palacios. 1992. Detection of specific antibodies in gingival crevicular transudate by enzyme-linked immunosorbent assay for diagnosis of human immunodeficiency virus type 1 infection. J. Clin. Microbiol. 30:2780–2783.
22. Zaabi, O., E. E. Machtet, H. Ben-Aryeh, L. Ardekian, M. Peled, and D. Laupner. 1999. The effect of smoking and periodontal treatment on salivary composition in patients with established periodontitis. J. Periodontol. 70:1240–1246.