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Reply

Peters and Cunningham raise questions that we also considered from the beginning of our studies of the proteins released by the LAN-1 neuroblastoma cell line. The claim that this cell line synthesizes proteins cross-reactive with BSA[1] is based on evidence in addition to that criticized by Peters and Cunningham: LAN-1 cells, grown in serum-free medium in which the only polypeptides are bovine insulin and human transferrin, release proteins that are precipitable by affinity-purified anti-BSA. These experiments were performed with culture supernatants from cells passaged more than 3 times at weekly intervals in the serum-free medium. In these, BSA is no longer detectable; yet, anti-BSA brings down numerous polypeptides not evident in the serum-free medium itself or in anti-BSA (Fig. 1; Ref. 2, p. 859).

Electrophoretic transfer of proteins from spent culture media to nitrocellulose paper after they had been fractionated by polyacrylamide gel electrophoresis also demonstrated that there was at least one protein, in addition to BSA, that reacted with anti-BSA. Finally, we showed that ^{125}I-labeled BSA did not form a complex with substances in the spent culture media that retards its passage in polyacrylamide gels under the conditions used in these experiments.

Peters and Cunningham were also concerned with our assertion that many of the children with neuroblastoma in our study had unusually high levels of antibody to BSA. They cite studies of Rothberg and Farr (3) as evidence that high levels of anti-BSA are quite prevalent in sera of both healthy and sick children less than 15 years old. There were important differences in the methods used to measure anti-BSA antibody in our studies and those of Rothberg and Farr. We used 50-μl aliquots of sera diluted 1:10; Rothberg and Farr used 2-ml aliquots of sera, also diluted 1:10. We added 0.15 μg; they added approximately 0.125 μg of labeled BSA to their test samples. Since we added approximately 48 times as much antigen to the same quantity of immunoglobulin as did Rothberg and Farr (3), sera from our patients had to contain proportionately more antibody in order to precipitate 10% of the added antigen. Under the conditions of our test, sera from 7 of the 17 neuroblastoma patients had higher anti-BSA levels than all but 3 of the healthy age-matched controls. In any case, the major reason for measuring the anti-BSA activity of these sera was to explore the relationship between these antibodies and the quantity of immune complexes detected by the Raji cell radioimmunoassay. The anti-BSA activity of the patient sera correlated significantly with the levels of immune complexes ($r_s = 0.54$; $p < 0.001$); moreover, sera that contained high levels of immune complex often contained hidden or "blocked" antibodies to BSA, suggesting that these contained anti-BSA bound to BSA or antigens that cross-react with BSA (1).

With regard to the question about the prevalence of breast feeding in our patient population, none had been breast fed for more than 3 months, and all had ingested cow's milk frequently before diagnosis of their tumor, as was reported (1).

Like Peters and Cunningham, we are fascinated by the results of these observations and agree that they should be pursued.

Daniel A. Beck
Centre Hospitalier Universitaire Vaudois
Service de Pediatrie
1011 Lausanne
Switzerland

Dwight B. DuBois
Roger D. Rossen[2]
The Veterans Administration Hospital and the Departments of Microbiology and Immunology, and Internal Medicine
Baylor College of Medicine
Houston, Texas 77211

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[1] The abbreviation used is: BSA, bovine serum albumin.

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[2] To whom requests for reprints should be addressed, at Building 211, Room 203, The Veterans Administration Medical Center, 2002 Holcombe, Houston, Texas 77211.