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Radioimmunoassay of Human Jejunal Iga Secretion[1] (38621)

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(Introduced by B. R. Forsyth)

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Secretory immunoglobulin-A (S-IgA) has been assigned a major protective role for a variety of epithelial surfaces. In the gastrointestinal tract, S-IgA inhibits adsorption of bacteria to mucosal cells and neutralizes viral antigens. Intestinal S-IgA is synthesized as a dimer in the lamina propria by immunocytes adjacent to epithelial cells, is conjugated with secretory component at the lateral membrane, enters the cell by pinocytosis, and is subsequently secreted into the lumen (1). Immunofluorescent studies indicate that S-IgA is synthesized throughout the gut. It has been estimated that there are from 350,000 to 980,000 IgA immunocytes per mm^3 of jejunal mucosa (2–5) and similar numbers in the ileum (6), colon (2), and rectum (2, 7). From 50% to 85% of gut immunocytes produce IgA (2, 4, 6). Jejunal fluid concentrations of S-IgA range from 21 to 41 mg/dl (7–11) but free secretory component has not been detected in jejunal aspirates (12). Eating, gallbladder contraction, pancreatic secretion, and swallowed IgA from oropharyngeal secretions alter intestinal concentrations (13). Recently, semiquantitative measurements of S-IgA synthesis have been reported in man (14) and synthesis and secretion have been documented in rabbits (15). To enable quantitative determination of human S-IgA secretion by intestinal mucosa, we developed a radioimmunoassay technique applicable to organ culture of jejunal biopsies from man.

Methods. Subjects. Five normal human volunteers between the ages of 40 and 57 gave informed consent for these investigations which were approved by the University of Vermont Committee on Human Experimentation. Upper G.I. and small bowel series, barium enema, sigmoidoscopy, rectal biopsy, quantitative jejunal bacterial cultures, ^{51}Cr-albumin clearance, quantitative fat balance, and small-bowel biopsies were normal in each subject.

Jejunal biopsies. Subjects were intubated with the Quinton hydraulic biopsy tube in the fasting state. Under fluoroscopic guidance, 8–10 biopsies were obtained from the region of the ligament of Treitz in each subject. A specimen was oriented on dehydrated cucumber and fixed in Bouin's solution for light microscopy and the remainder of the biopsies were processed for S-IgA secretion studies in organ culture.

Jejunal organ cultures. This system was used to determine incorporation of radioactive leucine into biopsy protein, secretion of radiolabeled protein into medium, and secretion of S-IgA. Intestinal biopsy samples were grown in organ culture according to the method of Browning and Trier (16) for 24 hr in the presence of ^{14}C-L-leucine, 2 μCi/ml (SA 305 mCi/mmole, New England Nuclear, Boston, MA). At the end of incubation, biopsies were removed from the medium and washed with chilled Hanks' solution containing 4.32 g/liter of unlabeled L-leucine. Biopsies were homogenized, and aliquots removed for protein determination and 10% TCA precipitation. Precipitates were centrifuged and resuspended in TCA, heated to 90°C for 10 min, iced, and collected on a Millipore filter (HAWP, 0.45-μm pore size, Millipore Filter Corp., Bedford, MA). TCA precipitates and filters were suspended in Aquasol (New England Nuclear, Boston, MA) and radioactivity was measured by liquid scintillation spectrometry. The results were expressed as cpm in biopsy protein.

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Medium and washes were combined and frozen at −70°C until use. Upon thawing, they were centrifuged at 6040 *g* for 10 min and the pellet discarded. To remove nonprotein radioactivity, the supernatant was diluted and concentrated repeatedly with phosphate buffer, pH 7, containing 0.15 *M* NaCl in an Amicon concentration chamber over a PM 30 membrane which had been saturated with bovine serum albumin to minimize adsorption of washed protein. When effluent radioactivity was less than twice background, the medium was concentrated to the original volume. An aliquot was precipitated with 10% TCA and the precipitate washed and monitored for radioactivity, which was expressed as total TCA precipitatable cpm in medium/mg biopsy protein. Additional aliquots were analyzed by immunoprecipitation for radioactivity incorporated into S-IgA and values expressed as percentage of cpm in medium present in S-IgA. Other aliquots of medium were analyzed quantitatively for S-IgA content by double precipitin radioimmunoassay.

Radioimmunoassay. Secretion of S-IgA by jejunal biopsies was quantified by radioimmunoassay utilizing the following proteins.

Colostral S-IgA, immunologically specific, was kindly supplied by W. R. Brown, M.D. of the University of Colorado. Radiolabeling was done with $Na^{125}I$ by the chloramine T method (17) followed by extensive dialysis.

Anti-human IgA goat antiserum, α chain specific, (Hyland Laboratories, Costa Mesa, CA) was immunologically pure by Ouchterlony immunodiffusion and immunoelectrophoresis against normal and a-gamma human serum. For use, the antiserum was diluted to 3.4 μg/ml, a concentration sufficient to precipitate 2 μg of colostral S-IgA, an amount in excess of that secreted in the volume of biopsy medium assayed.

Goat gamma globulin (Miles Laboratories, Inc., Kankakee, IL) which showed no cross reaction with human serum protein by Ouchterlony immunodiffusion, was added as carrier protein in a final concentration of 66.6 μg/ml.

Anti-goat gamma globulin antiserum, from Miles-Yeda Limited, Kiryat, Israel, 250 μg/ml, or from Cappel Laboratories, Downington, PA, 725 μg/ml, was used to precipitate the anti IgA-IgA complexes and carrier protein.

Standard curves were derived in assay systems containing from 0 to 0.713 μg of unlabeled colostral S-IgA, 25 μg of goat gamma globulin carrier, 0.034 μg goat antihuman S-IgA antibody, and 0.285 μg colostral ^{125}I-S-IgA (added last) brought to a final volume of 0.3 ml with 0.05 *M* phosphate buffer, pH 7, containing 0.15 *M* NaCl. Solutions were incubated for 30 min at 37°C, precipitated with 100 μg Miles-Yeda or 290 μg Cappel rabbit anti-goat gamma globulin, and held at 4°C for 24 hr. Samples were centrifuged at 6040 *g* for 30 min, the supernatants saved, and the precipitates were washed in iced phosphate–NaCl buffer and centrifuged again. Supernatants and washes were pooled and counted and pellets were dissolved in 1.5 ml 0.2 *N* NaOH for determination of radioactivity in a Nuclear Chicago gamma detector. To correct for nonspecific binding of S-IgA, radioactivity precipitated in assays free of specific goat antihuman IgA antibody was subtracted from immunoprecipitated radioactivity, and the results were plotted as percentage binding vs log concentration of unlabeled S-IgA. All assays were run in duplicate.

S-IgA in biopsy medium was assayed in duplicate in systems containing 10λ and 20λ of medium instead of unlabeled colostral S-IgA. Variation between duplicate samples was less than 10%. After subtracting blanks, values were read directly from the standard curve. Only values falling within the 10%–80% binding range were used. Values were corrected for dilution, the mean calculated, and data expressed as μg S-IgA/mg biopsy protein/hr.

Protein concentrations were determined by the method of Lowry *et al.* (18).

Results. Secretion of S-IgA by jejunal biopsies averaged 15 μg/mg of biopsy protein/24 hr as shown in Table I. Approximately 30% of the total radioactive protein in the medium was S-IgA. A reproducible standard curve is shown in Fig. 1. The method permits measurement of as little

as 0.015 μg of S-IgA in the incubation medium, and serial assays of the same medium at different times varied less than 10%. The method requires 24 hr for culture and 2 days for radioimmunoassay.

Discussion. This assay provides an accurate method for quantitative measurement of S-IgA secretion in 24-hr organ cultures of human jejunal biopsies. The stability of this system has been documented by Browning and Trier (16). We have found it reliable in animal studies (19) and in separate investigations of human biopsies; ^{14}C uptake was similar during the first and last hour of 24-hr incubations. Our radioimmunoassay technique assumes that the immunoprotein in the medium is structurally and immunologically similar to the colostral S-IgA which it is displacing in the assay system, and is not the IgA monomer. This assumption is supported by studies utilizing organ cultures of rabbit jejunum which indicate that IgA monomer secretion is negligible under these circumstances (15, 20), and by recent immunohistochemical investigations which show that monomer-producing immunocytes are rare in normal human intestinal biopsies (21).

TABLE I. S-IGA SECRETION BY JEJUNAL BIOPSIES.

Subject	Protein in biopsy (mg)	Biopsy protein (10^{-6} cpm)	Medium protein (10^{-5} cpm)	Medium S-IgA (% cpm)	S-IgA secreted per mg biopsy protein per 24 hr (mg)
WK	3.36	3.91	1.99	22.4	7.1
WM	3.60	3.71	3.64	30.6	33.2
WB	5.28	2.30	2.41	43.3	12.2
VR	4.57	2.40	3.50	30.1	17.3
MH	4.81	2.83	2.35	21.5	5.7
Mean	4.32	3.03	2.78	29.6	15.1
SD	.82	.74	.74	8.8	11.1
SE	.36	.33	.33	3.9	5.0

S-IgA in the medium represented 30% of the protein secreted during the 24-hr period, a value similar to the 18%–21% found in 12- to 24-hr incubations of rabbit jejunum (15, 20). In separate studies, measurements of S-IgA concentrations by immunodiffusion of intestinal fluid in WK, WM, and MH showed values of 5.3, 27.8, and 32.5 μg/dl, respectively. Because a variety of factors influence S-IgA concentration in jejunal aspirates, we would not expect these values to correlate closely with secretion rate. This relationship is currently under investigation.

The radioimmunoassay permits accurate measurements with four to eight jejunal biopsies, a number that can be obtained with acceptable safety. In addition, the 24-hr culture system minimizes the impact of the initial 4- to 6-hr lag period in protein

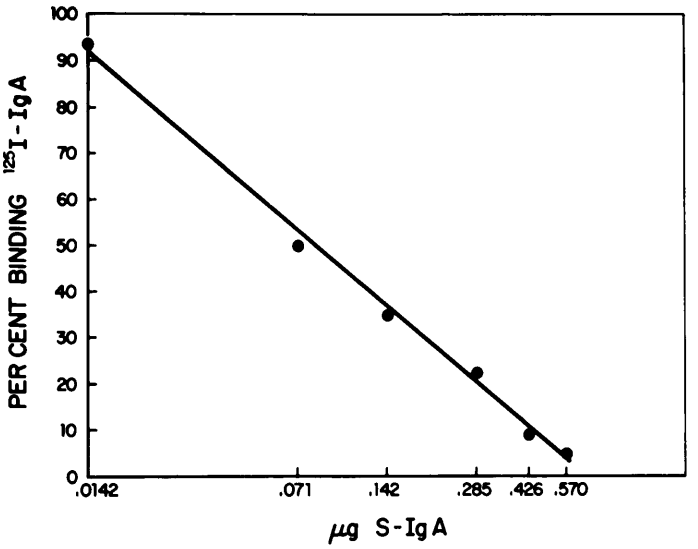

FIG. 1. Radioimmunoassay standard curve.

secretion observed by others (15, 20). Finally, our technique provides a sensitive method for studying human immunoglobulin A secretion under a variety of conditions which may be carefully manipulated in the organ culture environment.

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