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IgA subclasses in various secretions and in serum

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Two subclasses of IgA, IgA1 and IgA2, have been described (Kunkel & Prendergast, 1966; Feinstein & Franklin, 1966; Vaerman & Heremans, 1966). IgA1 predominates in serum, with reported mean serum IgA2 percentages of 23% ($n=3$; Grey, Abel, Yount & Kunkel, 1968), 11% ($n=24$; Morell, Skvaril, Noseda & Barandun, 1973) and 18% ($n=21$; Skvaril & Morell, 1974). The sole data for secretions are a higher IgA2 percentage in colostrum ($\bar{x}=45\%$; $n=4$) and saliva than in serum (Grey *et al.*, 1968). More recently, André, André & Fargier (1978) reported a higher proportion of IgA2 plasmacytes in secretory sites than in peripheral lymph nodes. Accordingly, IgA2 levels in serum, like those of polymeric IgA (p-IgA) have been used as an index of mucosal events (André, Berthoux, André, Gillon, Genin & Sabatier, 1980). As no other data exist on IgA subclasses in secretions, we measured IgA1 and IgA2 levels as well as the size distribution of IgA in various secretions and in serum.

The secretions studied are listed in Table 1. All of the samples were obtained from healthy Caucasian adults, except for bronchial secretions and hepatic bile which were obtained from patients undergoing endoscopy for the investigation of an extrapulmonary tumour or of a cryptogenic abdominal pain, respectively. The intestinal and bronchial secretions were collected by perfusion-washing of, respectively, the jejunum with an occluding balloon (Rambaud, Duprey, Novel, Hostein, Delpech & Bernier, 1981), and an upper segmental bronchus. Serum was obtained simultaneously with the secretions except for intestinal secretions which were compared with thirty-four healthy adult sera. Monomeric (m-IgA) and p-IgA were separated by sucrose density gradient ultracentrifugation (SDGU; Delacroix, Meykens & Vaerman, 1982c) of 4–150 μl of sample, diluted to 0·3 ml with buffered saline. Total IgA levels were measured in serum by immunonephelometry, and in secretions or in SDGU-fractions by immunoradiometric assays (IRMA) with correction for the underestimation of p-IgA in IRMA as described (Delacroix, Dehennin & Vaerman, 1982a). Subclass specific rabbit antisera were obtained after multiple injections of monoclonal IgA1 or IgA2, and solid phase absorption with IgA2 or IgA1 myeloma sera, respectively, of appropriate light chain type. The IgA1- and IgA2-IRMA (standard ranges: 2 to 30 ng/ml) measured less than 1% of three different monoclonal IgA2 and IgA1, of both light chain types, respectively (Fig. 1). All samples were assayed at several dilutions, which closely reproduced the standard curve below 30 ng/ml. A large pool of normal sera was used as standard for all assays: it contained 13% of p-IgA and 21% of IgA2

Abbreviations: p-IgA, polymeric IgA; m-IgA, monomeric IgA; SDGU, sucrose density gradient ultracentrifugation.

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Table 1. IgA subclass and size distribution in secretions compared to serum

Secretion	Total IgA (μg/ml) x̄ and range	p-IgA (%)	secret p-IgA / m-IgA serum	secret* serum	IgA2/(IgA1+IgA2), x̄±SD in Secretion		Serum
Saliva ($n=7$)	229 (87–615)	96	129		37±13	¶$p<0.02$	20±8
Intestinal ($n=11$)†	14 (4–48)	95	125		30±5	$p<0.01$	21±7**
Milk ($n=6$)‡	1631 (743–4561)	96	194		35±3	$p<0.05$	19±5
Tears ($n=6$)§	124 (39–381)	95	147		41±5	$p<0.05$	20±5
Bronchial ($n=6$)†	18 (4–70)	82	37		33±9	$p<0.05$	18±8
Hepatic bile ($n=11$)	79 (22–187)	65	12		26±8	$p<0.01$	21±6

* Calculated from analysis of pooled sample.
† Obtained by perfusion-washing.
‡ Collected between day 2 and 7 *post partum*.
§ Obtained after mechanical stimulation of the nasal mucosa.
¶ Wilcoxon signed rank test.
** Sera from thirty-four healthy adults, compared by Mann Whitney U test.

when referred to purified monoclonal IgA1 and IgA2 proteins assayed by total IgA-IRMA. IgA2 percentages (IgA2 × 100/IgA1 + IgA2) were compared in secretions and serum by the Wilcoxon rank test for paired values, or by the Mann-Whitney U test for intestinal secretions.

Total IgA levels, percentages of p-IgA, secretion-to-serum concentration ratios for p-IgA expressed relatively to those for m-IgA, and IgA2 percentages in secretions and sera are listed in Table 1. Only bile and bronchial secretions contained a substantial proportion of monomer. All secretions contained significantly more IgA2 than their corresponding serum, with highest proportions in tears, saliva and milk. The proportion of IgA2 was larger in p-IgA (35%) than in m-IgA (25%) for SDGU-fractionated bile, but similar proportions of IgA2 were found in serum m-IgA (22%) and p-IgA (23%) when separated by SDGU (not shown). The ratio (IgA1 + IgA2): total IgA averaged 1·11 ± 0·04 in secretions, and 1·05 ± 0·06 in sera.

Our data generalize the existence of higher IgA2 percentages in secretions than in serum. The selectivity of secretion of p-IgA versus m-IgA was lower in bronchial secretions and in bile than in saliva, milk, tears and intestinal secretions. Bile displayed the lowest IgA2 percentage, probably because as much as 50% of p-IgA and 82% of m-IgA in bile derive from serum, as compared with 1·6% and 30%, respectively, in saliva (Delacroix, Hodgson, McPherson, Dive & Vaerman, 1982b). Although IgA2 and p-IgA predomi-

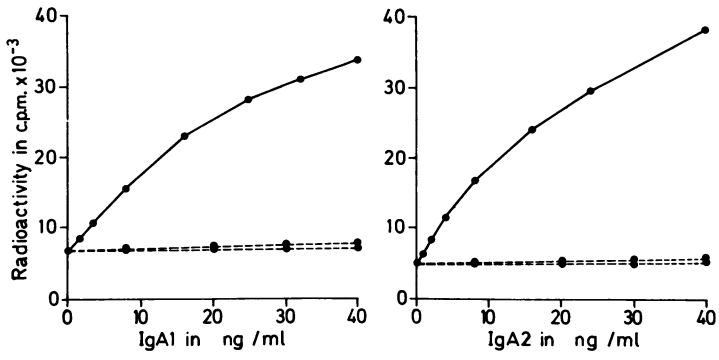

Figure 1. Standard curves for the immunoradiometric assays of IgA1 (left) and IgA2 (right). Dotted lines are measurements of two IgA2 (one λ, one κ) at left, and of two IgA1 (one λ, one κ) at right.

nate together in secretions, our results on subclass distribution in separated serum m- and p-IgA showed similar proportions of IgA2 in both size forms, indicating that the two molecular characters, IgA2 and p-IgA, are not necessarily linked. This is in agreement with our recent observation of high proportions of p-IgA without shift of the subclass distribution to IgA2 in sera of infants (Delacroix, Liroux & Vaerman, submitted for publication).

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