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Equine Secretory IgA and Secretory Component

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Abstract. A complete secretory immunologic system has been identified in the equine species. It is characterised by the presence of a secretory component either bound to secretory IgA (SIgA) or remaining in the free form (FSC). The mean molecular weights of SIgA, serum IgA and FSC have been estimated. The homology of the equine and human IgA classes have been demonstrated by cross-reaction with anti-human IgA antisera. A quantitative study of equine immunoglobulins in various fluids have shown that SIgA is predominant in saliva, mature milk, nasal and lacrimal secretions, but not in colostrum. *In vitro* binding of human and bovine FSC is found to occur mostly with the polymeric form of equine serum IgA.

Introduction

The identification of equine IgA has been for a long time a controversial subject. The horse T-globulin identified by VAN DER SCHEER and WYCKOFF [19] has been described as being IgA by several authors [4, 14]. However, this fast γ-globulin is now considered to be an IgG subclass called IgGT, because its heavy chain shares many common antigenic determinants with other known equine IgG subclasses (IgG a, b, c). Its C-terminal amino acid sequence presents definite homology with these equine IgG as well as with human and rabbit IgG [20]. Immunoglobulins in equine secretions have been investigated only recently. In a study of colostral and salivary secretions, GENCO *et al.* [4] concluded that 'there appears to be little or no secretory γA-immunoglobulin in equine parotid fluid or colostrum that is comparable in size and antigen-

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icity to that found in some other mammals'. Previously, AUDIBERT and SANDOR [1] had purified a colostral protein of fast electrophoretic mobility soluble in zinc sulphate and with a high carbohydrate content. This protein, immunologically distinct from the T-globulin, was called IgA. The first proof of homology between human IgA and a horse serum and milk protein was given recently by VAERMAN *et al.* [17, 18] using serological cross-reactions. However, these authors were unable to demonstrate the presence of specific antigenic determinants on the IgA from the secretions.

The purpose of this work is: (a) to prove the existence of an equine secretory immunologic system characterised by a secretory component (SC), either bound to IgA or in a free form (FSC), which is comparable to the one described in other species [2, 3, 7, 8, 10, 11, 13, 16]; (b) to make a quantitative study of this newly described immunoglobulin in various fluids compared to the known IgG and IgM, and (c) to test the ability of equine serum IgA to bind human or bovine FSC *in vitro*.

Materials and Methods

Samples. The 15 bloods, 6 colostra, 6 mature milks, 6 salivas, 6 lacrimal secretions, 6 nasal secretions and 3 spermatic fluids were obtained thanks to the courtesy of Dr. LEUENBERGER at the 'Haras Federal Suisse', Avenches.

Preparation of immunoglobulins. Purification of equine immunoglobulins was performed by ammonium sulphate precipitation, ion exchange chromatography and gel filtration, as previously described for the bovine species [7]. Colostral secretory IgA (SIgA) was first precipitated from colostrum whey with ammonium sulphate at 40% saturation (room temperature, pH 7.4). The precipitate was dissolved in PBS, exhaustively dialysed against phosphate buffer 0.01 M, pH 7.4 (KH_2PO_4-Na_2HPO_4), and applied to a DEAE-cellulose column equilibrated with the same buffer. Slow IgG fractions were removed with buffer 0.02 M, pH 7.4, and colostral SIgA was eluted with buffer 0.06 M, pH 7.0. Upon filtration of this material on Sephadex G-200 the SIgA elutes between the exclusion peak and the main 7S fraction (fig. 2A). A second Sephadex G-200 filtration yielded pure SIgA.

In serum, IgA appears in two forms, probably polymers (mostly dimers) and monomers, separable by gel filtration (fraction I and II; fig. 2C and 4B). For the purification of serum IgA, the starting material was a pseudoglobulin fraction, obtained from a 40-percent ammonium sulphate precipitate dialysed against a phosphate buffer 0.005 M, pH 6.0. Most of the monomeric form of serum IgA was eluted from DEAE-cellulose with a phosphate buffer 0.06 M, pH 7.0, while the polymeric form was eluted with a buffer 0.15 M, pH 6.0. IgM was purified from the euglobin fraction precipitated during the preparation of serum IgA. The fractionation scheme described for the bovine IgM was used [7]. A crude fraction of FSC

was prepared from mature milk by an ammonium sulphate precipitation at 50% saturation and a filtration on Sephadex G-200. The FSC is eluted in a zone corresponding to the descending limb of the main IgG peak (fig. 2B). This fraction was used for double diffusion analysis.

A pool of 7S IgG to be used as standard for the quantitation experiments was prepared from sera with a low level of IgA. An ammonium sulphate precipitate of these sera at 33% saturation was dissolved, dialysed against PBS, and filtered on Sephadex G-200. The fractions corresponding to the descending limb of the 7S peak were selected in order to avoid contamination with IgA, and refiltered on Sephadex G-200. This preparation showed only IgG lines when tested by polyvalent antisera on immunoelectrophoresis.

Antisera. Specific antisera to human and bovine IgA, IgM and IgG were used [7]. Specific antisera against the equine IgA and IgM were induced by injection of 1 mg of purified protein every 10 days, over a period of 2 months; subsequently they were rendered specific by absorption with fractions free of the immunoglobulin class being studied. Cross-precipitates with anti-human IgA sera were also prepared and injected into rabbits. The antisera thus obtained were monospecific when absorbed with IgG to remove anti-light chain antibodies. A specific antiserum against SC was obtained by absorption of an anti-SIgA antiserum with whole equine serum in order to remove anti-IgA antibodies (1 ml antiserum with 0.2 ml equine serum).

Protein concentration. Concentrations of specific immunoglobulin classes were estimated by the single radial immunodiffusion technique using cross-reacting anti-bovine sera or specific antisera against the equine immunoglobulin [10]. Concentration of protein in standard preparations was determined by micro-Kjeldahl and optical density at 280 nm.

Binding of human and bovine free secretory components. The technique for the study of FSC binding has been described in an earlier publication [6]. Briefly, 100 μg of the purified ^{125}I-labelled FSC in PBS were incubated for 1 h at 37 °C, either with 1 ml of equine serum or with 8 mg of the fractions I or II, containing IgA polymers and monomers (fig. 4B). After incubation, the mixtures were filtered on Sephadex G-200. Protein distribution was determined by absorbance measurements at 280 nm, and the distribution of the labelled FSC monitored in a scintillation counter. The specificity of FSC binding was determined by autoradiography following immunoelectrophoresis of the incubated serum and fractions I and II.

Results

The common antigenicity of equine and human IgA has been demonstrated by cross-reaction as described for several mammalian species [7, 10, 13, 17, 18]. Specific antisera against human SIgA and α-chain are found to cross-react with a protein of the same electrophoretic mobility in secretions and serum (fig. 1A–C). In double diffusion analysis, a partial identity is shown between the equine and human IgA when tested

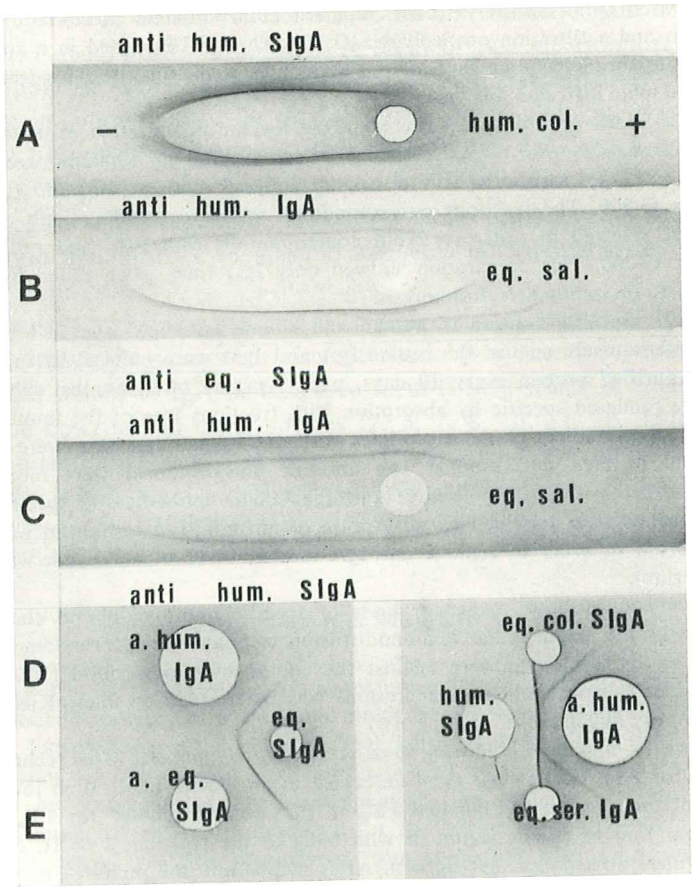

Fig. 1. Immunoelectrophoresis and double diffusion analysis demonstrating cross-reactivity of rabbit anti-human IgA with equine secretory and serum IgA. Abbreviations: anti hum. SIgA = antiserum specific for human α-chain and secretory component; anti hum. IgA = antiserum specific for human α-chain; anti eq. SIgA = antiserum specific for equine α-chain and secretory component; hum. col. = human colostrum whey; eq. sal. = equine saliva; eq. col. SIgA = equine colostral secretory IgA; eq. ser. IgA = equine serum IgA.

against an anti-human α-chain serum (fig. 1E). A species-specific anti-equine SIgA serum is obtained by immunisation of rabbits with a purified SIgA fraction. When this antiserum is tested together with the cross-reacting anti-human IgA against equine saliva or serum, they react with the same protein, giving a complete fusion of the precipitin lines in dou-

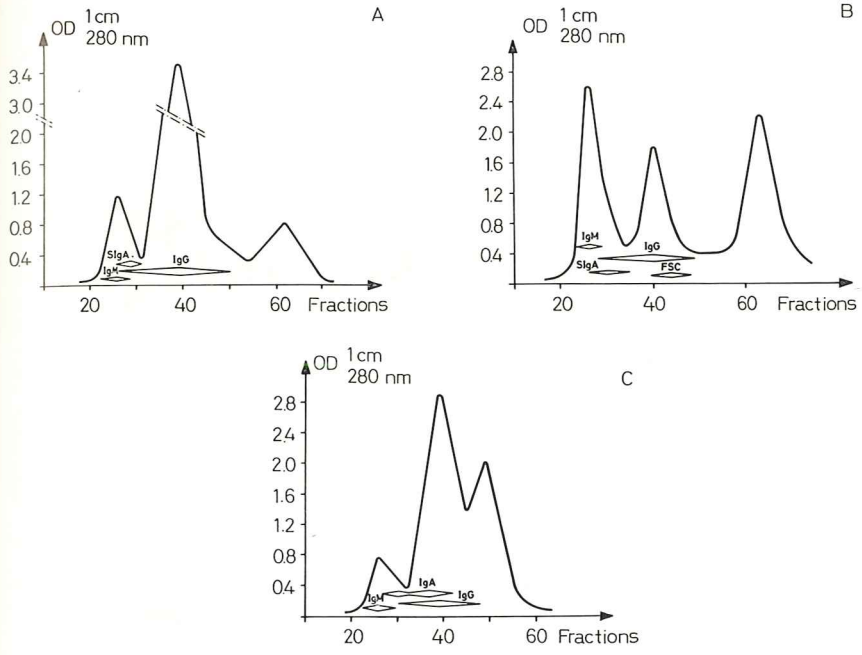

Fig. 2. Sephadex G-200 filtration *A* of whole equine colostrum whey; *B* of a 50-percent saturation ammonium sulphate precipitate from mature milk whey; and *C* of whole serum (column of 2.5 cm diameter × 110 cm length). Determination of the zones of elution of immunoglobulins and free secretory component (FSC) by the single radial immunodiffusion technique.

ble diffusion and the same γ-line on immunoelectrophoresis (fig. 1D, 1B and 3A–C). Furthermore, in mature milk, saliva, lacrimal and nasal secretions, the species-specific anti-SIgA reveals an additional β_2-line fusing with the γ-line at their cathodic end (fig. 1B, 3A and C). This β_2-protein proves to be the free secretory component (FSC) of the equine species. It is not precipitated by cross-reaction with an anti-human SIgA antiserum (fig. 1C). It is not demonstrable in serum and present only as traces in colostrum. Its elution volume by filtration on Sephadex G-200 (fig. 2B) corresponds to a molecular weight of about 80,000 comparable to the value found by the same method for the homologous protein in different species [8–10]. By double diffusion analysis, a FSC-rich fraction gives a line of partial identity with SIgA (fig. 3E). Using the same technique and the same antiserum, the presence of the secretory compo-

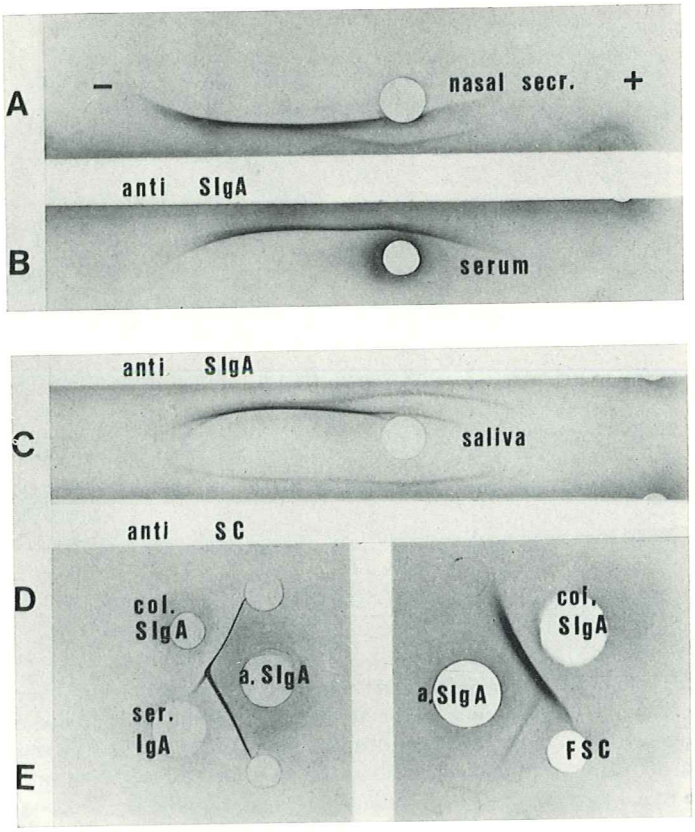

Fig 3. Immunoelectrophoresis and double diffusion analysis of equine secretions and serum demonstrating the presence of a secretory component either bound to IgA or in the free form. Abbreviations: anti SC = antiserum specific for equine secretory component; nasal secr. = nasal secretions; other abbreviations as in figure 1.

nent on equine SIgA is demonstrated by its classical spur over serum IgA (fig. 3D). Horse SIgA has the same behaviour as bovine SIgA on ion exchange chromatography and gel filtration (fig. 2A–B) and it is purified from colostral whey following the same scheme of fractionation [7]. The determination of its molecular weight by gel filtration gives a value of 400,000 ± 50,000.

Equine immunoglobulins are quantitated in serum, colostrum, mature milk, saliva, spermatic fluid, nasal and lacrimal secretions. Mean and

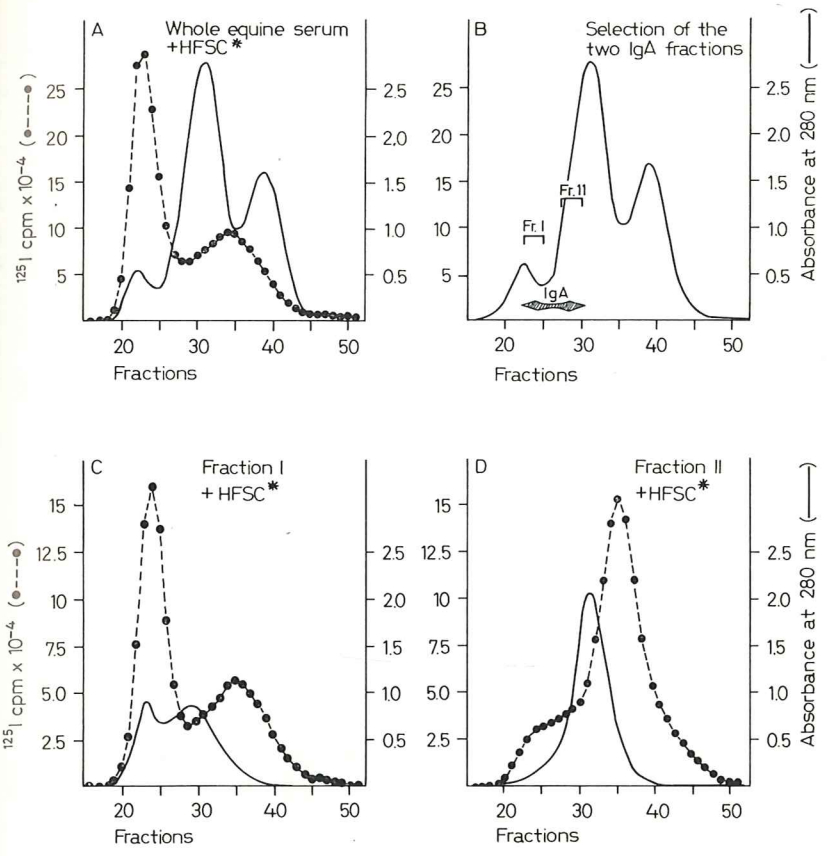

Fig. 4. Sephadex G-200 filtration analysing the combining ability of ^{125}I-labelled human secretory component (HFSC*) with *A* whole equine serum; *C* polymeric serum IgA; *D* monomeric serum IgA. In *B*, separation of the two IgA populations. Protein distribution determined by optical density at 280 nm is indicated by the continuous line (—) and the radioactive cpm by the dashed line (---).

limit values are given in table I. It must be recalled that all secretions were obtained without stimulation and that no previous concentration was necessary. The different IgG subclasses which have been already extensively studied [1, 4, 14, 15, 20] are not quantitated separately.

In serum, two IgA populations of different molecular weights are identified by filtration on Sephadex G-200 (fig. 2C). Their elution volumes correspond to molecular weights of 175,000 ± 12,000 and

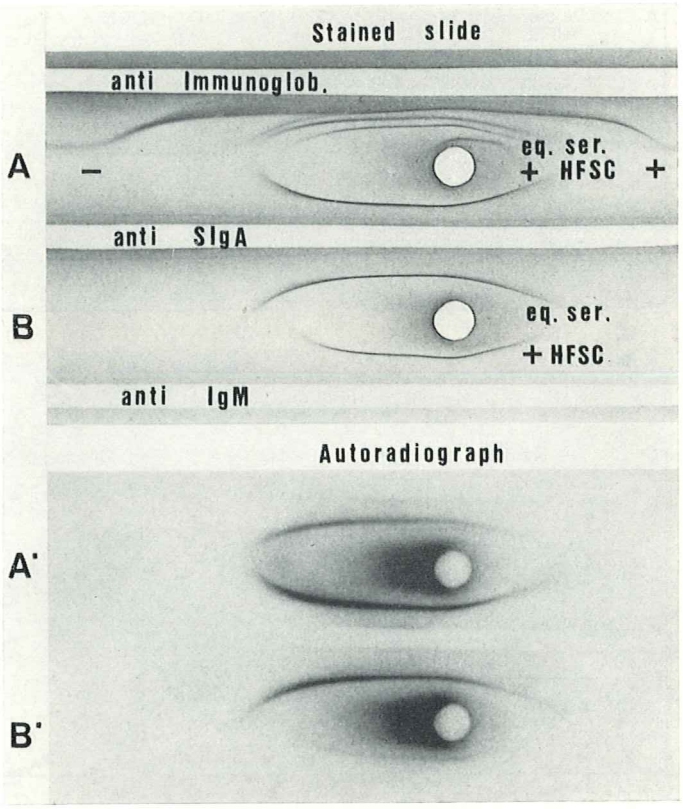

Fig. 5. Immunoelectrophoresis (A-B) and autoradiography (A′ – B′) of equine serum immunoglobulins showing the combination of ^{125}I-labelled human free secretory component with polymeric IgA and IgM. The wells contain equine serum following incubation with ^{125}I human free secretory component. Rabbit antisera against all equine immunoglobulins or monospecific for equine SIgA and IgM are used as indicated in the picture.

340,000 ± 40,000. These values suggest that serum IgA exists mainly as monomers and dimers. We took advantage of the presence of these two populations of IgA in equine serum to investigate their respective ability to bind human or bovine FSC *in vitro*. First, ^{125}I-labelled human FSC (HFSC*) is incubated with whole equine serum. Immunoelectrophoresis and autoradiography demonstrate that the labelled FSC is bound mostly to IgA, in a much smaller degree to IgM, but not at all to IgG (fig. 5).

Table I. Immunoglobulin levels in equine external secretions and serum[1]

Sample	Protein concentrations, mean and limit values, mg/100 ml		
	IgA	IgG	IgM
Lacrimal secretions	200 (100–400) 93 %[2]	12 (5–20)	3 (2–4)
Nasal secretions	160 (80–300) 95 %	8 (5–10)	traces
Saliva	120 (40–300) 88 %	15 (5–20)	1.5 (1–2)
Spermatic fluid	8 (4–10) 67 %	4 (2–5)	traces
Colostrum	900 (500–1,600) 10 %	8,000 (6,000–9,000)	400 (160–600)
Milk	80 (50–120) 67 %	35 (20–50)	4 (2–5)
Serum	200 (80–300 9 %	1,800 (1,600–1,900)	180 (160–200)

[1] Immunoglobulin levels were determined by the single radial immunodiffusion technique (see Materials and Methods).

[2] Percentage of IgA in total immunoglobulin.

Filtration of the incubated protein on Sephadex G-200 shows that 70% of the labelled FSC is eluted at the exclusion volume (fig. 4A). Autoradiography of this fraction confirms the binding with IgA. Then the two populations of serum IgA are tested for their combining ability with FSC after separation on Sephadex G-200 (fig. 4B). Most of the labelled FSC (65%) is bound by the polymeric IgA (fraction I), but only a small amount (10%) by the monomeric form (fraction II) (fig. 4C-D). It is likely that this slight combination with the monomeric IgA fraction is due to contamination with polymers (the two IgA fractions were separated by only one gel filtration). Thus, the polymeric form of IgA is necessary for the *in vitro* combination. The results obtained with the labelled bovine FSC do not differ markedly and lead to the same conclusions.

Discussion

Although one of the best criteria for the identification of an IgA class in a newly studied species is assumed to be the serological cross-reactivity with human IgA [17, 18], the existence of a secretory immunologic system can be established only by the demonstration of the presence of specific antigenic determinants on the SIgA and by the identification of

a free secretory component showing partial identity with SIgA. This free form of SC has been immunologically characterised in man, cow, goat, sheep and dog [7–10, 13]. Due to its poor immunogenicity the secretory component can be demonstrated only after a prolonged immunisation of rabbits with SIgA.

Equine SIgA had been partially identified and purified by AUDIBERT and SANDOR [1]. In our hands, an anti-colostral IgA serum produced in their laboratory reveals the same protein as our specific antisera, reacting also with the FSC. However, at the time of their investigations, these authors did not use serological cross-reactions to ascertain the homology of their equine immunoglobulin with the human IgA and they did not observe the presence of additional secretory determinants on colostral IgA compared to serum IgA.

The quantitative study shows that while SIgA is a relatively minor component of colostrum compared to the large amount of IgG, it is the major immunoglobulin in most other secretions like mature milk, saliva, nasal and lacrimal fluids. Similar results were observed in ruminants such as cow, sheep and goat, which transfer passive immunity to their offspring by the colostrum essentially in the form of IgG. On the other hand, species like human, monkey or rabbit, able to transfer IgG to their foetus through the placenta, have a colostrum containing mostly SIgA. Thus this quantitative study of equine immunoglobulins confirms our hypothesis that there is a correlation between the type of immunoglobulins in colostrum and the mode of transfer of passive immunity [7].

The binding of the human and bovine FSC mostly with the polymeric form of the equine serum IgA parallel the observation made in the case of human IgA myelomas [6, 12]. The specific ability of the IgA polymers to bind FSC can be interpreted in view of the recent work of HALPERN and KOSHLAND [5]. These authors demonstrated the presence of an additional polypeptide chain on IgA polymers and suggested that this 'J-chain' would be responsible for the linkage of the monomers and for the binding of the SC on SIgA. If their hypothesis is correct our experiments would then indicate that the 'J-chain' is present on equine IgA polymers. The same structure could be assigned to the IgA and IgM of 8 mammalian species previously studied [6].

In addition to the serological cross-reactivity between human and equine IgA, the cross-combination of the human FSC with equine serum IgA and the demonstration of an equine SC emphasise the marked homology between the secretory immunologic systems of these two species.

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