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The effect of aging on the secretory immune system of the eye

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SUMMARY

The objective of the present study was to examine the influence of aging on the ocular secretory immune system of the eye. Levels of IgA and free secretory component (FSC) were measured in lacrimal glands and/or tears of 0·6, 1·3, 3, 8 and 17-month-old male and female rats. In addition, the FSC output of lacrimal tissue cultured *in vitro* was evaluated. During the period from 0·6 to 1·3 months of age, the content of tear IgA increased nine- and 13-fold in females and males, respectively. This rise was paralleled by changes in the concentration of tear FSC. Prior to the onset of puberty, FSC could be detected in only 7% of tear samples, whereas after pubertal maturation, tear FSC levels had attained adult concentrations. This tear FSC profile was similar to the age-related pattern of FSC output by lacrimal tissue incubated *in vitro*. Following puberty, tear IgA content continued to increase in both sexes until adulthood (3 months of age) and then plateaued in females from 8 to 17 months of age. In contrast, tear IgA in males appeared to stabilize from 3 to 8 months and then rose significantly to the highest levels at 17 months of age. This increase in males was also reflected in their lacrimal tissue: IgA content underwent a six-fold elevation from 3 to 17 months. Of interest is that the differential kinetics involved in tear IgA and FSC expression resulted in an age-associated decline in the FSC/IgA ratio from post-puberty to senescence. A striking finding in these studies was the persistence of a sexual dimorphism in the secretory immune system of the eye. After pubertal development, IgA and FSC levels were significantly higher in tears of males, compared to those of females, at all ages tested up to 17 months. These gender- and age-related variations in tear IgA and FSC amounts could not be accounted for by changes in either the volume of, or total protein content in, tears.

INTRODUCTION

Previous research has demonstrated that aging is accompanied by a progressive degeneration of lymphoid tissue, a marked dysfunction in systemic immunity and an increase in the incidence of infectious and autoimmune disease (Makinodan & Kay, 1980; Wade & Szewczuk, 1984). These age-related immunological sequelae appear to be due in part to profound alterations in both B- and T-cell compartments. Thus, in senescent animals, lymphocytes exhibit diminished function, decreased response to antigenic challenge and reduced proliferative capacity (Makinodan & Kay, 1980; Wade & Szewczuk, 1984).

This impact of aging may also extend to the secretory immune system, which protects mucosal surfaces against microbial infection (Ganguly & Waldman, 1980; Brandtzaeg, 1985). Studies have shown that senescence is associated with decreased concentrations of IgA in nasal (Alford, 1968) and intestinal (Lim, Messiha & Watson, 1981) secretions, reduced hepatic clearance of IgA antibodies (Schmucker *et al.*, 1985), attenuated salivary IgA response to antigenic exposure (Smith, Ebersole & Taubman, 1983), and a dramatic decline in the density of IgA-containing cells in the mesenteric lymph nodes (Haaijman & Hijmans, 1978).

The purpose of the present study was to determine whether aging might also influence the secretory immune system of the eye. Our experiments, which utilized infant, pre-pubertal, post-pubertal, adult, mid-life and senescent rats, were designed to: (i) examine the effect of aging on the levels of IgA and free secretory component (FSC) in tears and lacrimal gland tissue; and (ii) assess whether gender might modify any impact of aging on ocular immunity, since the secretory immune system of adult (2–3-month-old) rats is known to be sexually dimorphic (Sullivan, Bloch & Allansmith, 1984a,b; Sullivan & Allansmith, 1985; Sullivan *et al.*, 1986). Our focus on the lacrimal gland in these studies, compared to other tissues of the ocular adnexa, was because this organ represents the primary source of both IgA (Brandtzaeg, 1985; Allansmith *et al.*, 1976; Sullivan & Allansmith, 1984; Gudmundsson *et al.*, 1985) and secretory component (SC) (Sullivan et al., 1984b; Gudmundsson *et al.*, 1985; Allansmith & Gillette, 1980) in tears. Lastly, for comparison with tear results, we evaluated whether aging influences the concentration of IgA and FSC in saliva of male and female rats.

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MATERIALS AND METHODS

Animals

Male and female Sprague–Dawley rats were obtained at 0·2 (6 days; infant), 0·6 (2·5 weeks, pre-pubertal), 1·3 (5·5 weeks, post-pubertal), 3·0 (adult), 8·0 (mid-life) and 17 (senescent) months of age from Zivic-Miller Laboratories Inc. (Allison Park, PA). Animals were maintained in constant-temperature rooms with light/dark intervals of 12 hr duration.

General procedures

Tears were collected from the eyes of ether-anaesthetized rats, as described previously (Sullivan *et al.*, 1984b; Sullivan & Allansmith, 1986). Briefly, the tip of a graded microcapillary pipet (5 μl capacity) was placed at the inner canthus and gently moved along the palpebral conjunctiva. This procedure was repeated twice on each eye, typically lasted less than 1 min, and drained the entire available tear volume. Tear volumes were accurately measured (to 0·1 μl), and then transferred to polypropylene tubes containing 100 μl 0·1 M sodium phosphate buffer, pH 7·0 (PB7). Immediately after the tear collection, rats were injected subcutaneously with pilocarpine nitrate (0·5 mg/100 g body weight) to stimulate the flow and permit the collection of saliva (Sullivan & Wira, 1983b). Blood was obtained from the tail and allowed to clot at room temperature. Samples were centrifuged for 4 min at 10,000 ***g***, and tear, salivary and serum supernatants were stored at −20° until use.

To permit the analysis of IgA, free secretory component (FSC) and protein levels in lacrimal gland tissue, glands were perfused and tissue cytosols prepared by the following reported protocols (Sullivan *et al.*, 1984a; Sullivan & Wira, 1983a). Briefly, the rats were killed and the ocular vasculature perfused *in situ* with excess 0·9% saline to remove residual blood. The descending aorta was cannulated below the diaphragm with a 19-gauge needle, which was attached by tubing to a raised saline reservoir. Perfusion was directed towards the heart, under which condition the aortic valve was shut and flow traversed the ascending aortic branch. The right atrium and ventricle were severed to allow the escape of venous fluid. After 50–100 ml of saline had been perfused, lacrimal (exorbital) glands were removed, rinsed in saline, blotted on surgical gauze, weighed and homogenized in 6 volumes of ice-cooled TKM buffer (50 mM Trizma, 25 mM KCl, 5 mM $MgCl_2$, pH 7·5). Homogenization was accomplished with a Polytron PT-10 (Brinkmann Instruments, Westbury, NY) by three 10-second bursts with 50-second intervals between bursts. Homogenates were centrifuged twice at 10,000 ***g*** for 4 min/centrifugation and supernatants (cytosols) volumetrically measured then stored at −20°.

To allow the measurement of FSC secretion by lacrimal tissue *in vitro*, lacrimal glands were removed and cultured according to procedures reported elsewhere (Sullivan *et al.*, 1984a; Sullivan & Allansmith, 1984, 1985). In brief, lacrimal glands were removed, rinsed in saline, blotted dry, quartered and weighed. Half of one tissue/rat was placed in 20-ml glass vials containing 2 ml of incubation medium, which consisted of RPMI-1640 with glutamine (Gibco, Grand Island, NY), 10% fetal bovine serum (Gibco), garamycin (25 μg/ml; Schering Corp., Bloomfield, NJ) and added glucose. Vials were gassed with 95% O_2–5% CO_2, capped and transferred to a shaking rack in a 37° water bath. After 22 hr of incubation, which included a period of vial regassing, media were removed, centrifuged at 10,000 ***g*** and supernatants stored at −20°.

Protein levels in tears, saliva, serum and lacrimal gland cytosol were measured by the Hartree (Hartree, 1972) method, utilizing bovine serum albumin (Calbiochem-Behring Corp., La Jolla, CA) as the standard. Statistical analysis of the data was performed with the Student's *t*-test.

Immunochemical procedures

The IgA and FSC levels in experimental samples were measured with specific radioimmunoassays (RIA), according to previously described techniques (Sullivan & Allansmith, 1984; Sullivan & Wira, 1983b). The assay for SC detected primarily free SC (FSC; Sullivan & Wira, 1983b), and therefore results have been reported in terms of FSC. Antigenic standards in the RIAs were affinity-purified rat polymeric IgA (a gift from Dr J. P. Vaerman, Catholic University of Louvain, Brussels, Belgium) or affinity-purified rat SC (a gift from Dr C. R. Wira, Dartmouth Medical School, Hanover, NH, and prepared by Dr B. Underdown, McMaster University, Ontario, Canada). These antigens were iodinated with IODO-GEN (Sullivan & Wira, 1983b) for use as tracer proteins. Unlabelled free SC and rat reference serum (Miles Laboratories, Elkhart, IN; containing 243 μg IgA/ml) were utilized to establish standard curves with each assay. Antisera included goat anti-rat IgA (Miles Laboratories) and rabbit anti-rat SC (from Dr Wira, prepared by Dr Underdown) as first antibodies, and rabbit anti-goat IgG (Miles Laboratories) and goat anti-rabbit IgG (Miles Laboratories) as second antibodies. The RIA sensitivities, defined as the lowest amount of antigen measurable and equal to the quantity 2SD above the zero dose response, were 3·3 ng IgA (Sullivan & Allansmith, 1984) and 0·44 ng FSC (Sullivan & Wira, 1983b).

RESULTS

Influence of age on the level of IgA in tears and serum of male and female rats

To evaluate whether age may influence the level of tear IgA, male and female Sprague–Dawley rats ($n = 6$–13/gender/age) were obtained at 0·2, 0·6, 1·3, 3·0, 8·0 and 17 months of age. Tear collection was performed once on all rats, except those in the 0·2-month age group: the ocular development in these 6-day-old animals had not sufficiently matured and their lids were still closed.

The impact of age on the tear IgA content is shown in Fig. 1. Before the onset of puberty (0·6 months), tear IgA levels were low and not significantly different between male and female rats. Within 3 weeks, however, the content of tear IgA underwent a considerable rise. During this period, which spanned much of puberty, tear IgA amounts increased approximately nine- and 13-fold in female and male rats, respectively. Moreover, the extent of this increase was gender dependent: tears of 1·3-month-old male rats had significantly ($P < 0·005$) higher levels of IgA than did those of age-matched females. From 1·3 to 3 months of age, tear IgA content in female rats continued to rise significantly ($P < 0·0005$), but then plateaued from 8 to 17 months of age. In contrast, tear IgA levels in males increased two-fold from 1·3 to 3 months, appeared to stabilize up to 8 months of age, and then rose by an additional four-fold amount at 17 months. At 3, 8 and 17 months of age, the content of tear

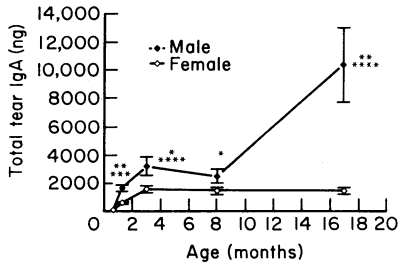

Figure 1. Influence of age on tear IgA levels in male and female rats. Tears were collected from 0·6, 1·3, 3·0, 8·0 and 17-month-old male and female rats ($n=6$–13/gender/age) and the total amount of IgA (ng) in each tear sample was determined. Points represent the mean ± SE. * Significantly ($P<0·05$) higher than value of age-matched female group; ** significantly ($P<0·005$) greater than value of age-matched female group; *** significantly ($P<0·001$) higher than the value of previous gender-matched age group; **** significantly ($P<0·05$) greater than value of previous gender-matched age group.

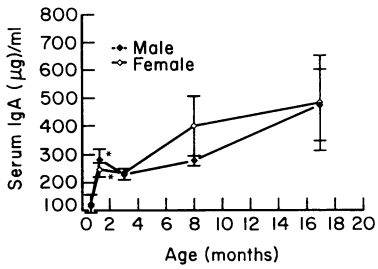

Figure 2. Effect of age on the serum IgA concentrations in male and female rats. Serum was obtained from 0·6, 1·3, 3·0, 8·0 and 17-month-old male and female rats ($n=6$–7/gender/age). Points equal the mean ± SE. * Significantly ($P<0·01$) greater than value in previous gender-matched group.

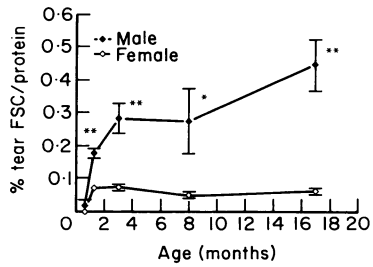

Figure 3. Impact of age on the tear FSC content in male and female rats. Tears were obtained from 0·6, 1·3, 3·0, 8·0 and 17-month-old male and female rats ($n=6$–13/gender/age). Levels of FSC (μg) in tears are expressed as a percentage of total tear protein (μg). Points represent the mean ± SE. * Significantly ($P<0·05$) higher than value of age-matched female group; ** significantly ($P<0·005$) greater than value of age-matched female group.

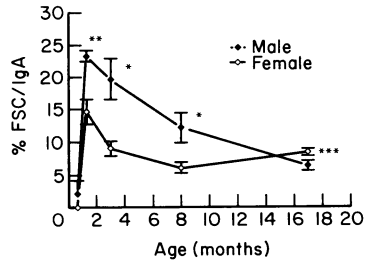

Figure 4. Influence of age on the FSC/IgA ratio in tears of male and female rats. Tears were collected from 0·6, 1·3, 3·0, 8·0 and 17-month-old male and female rats ($n=6$–13/gender/age). Levels of FSC (μg) in tears are expressed as a percentage of tear IgA (μg). Points equal the mean ± SE. * Significantly ($P<0·05$) higher than value of age-matched female group; ** significantly ($P<0·005$) greater than value of age-matched female group; *** significantly ($P<0·05$) higher than value of age-matched male group.

IgA in male rats was also significantly ($P<0·05$) greater than that of female rats.

These age-associated changes in tear IgA levels could not be accounted for by variations in tear volume. Tear volumes increased by factors of 5 and 8 in female and male rats, respectively, from 0·6 to 17 months of age. However, during the same interval, tear IgA contents rose by 22- and 84-fold amounts in females and males, respectively.

Similarly, the effects of age or gender could not be explained by fluctuations in total tear protein. Protein levels in tears increased approximately six-fold from 0·6 to 3 months of age, whereas IgA content rose by a 25-fold factor. Consequently, when expressed as a percentage of total protein, tear IgA levels increased from 0·39 ± 0·11% to 2·3 ± 0·76% in males and 0·25 ± 0·12 to 0·93 ± 0·12% in females during that time period. Moreover, there was no additional elevation in total tear protein from 3 to 17 months of age and no gender-related differences in total tear protein at these ages. Thus, at 17 months of age, the IgA/protein ratio in tears of male rats (8·98 ± 2·35%) was significantly ($P<0·005$) higher than that in tears of female rats (0·80 ± 0·15%).

In serum, the concentration of IgA increased in male and female rats ($n=6$–7/gender/age) from 0·6 to 1·3 months of age, but did not change significantly beyond this time (Fig. 2). No gender-related differences in the serum IgA concentration were found at any age examined.

Effect of age on the FSC levels and FSC/IgA ratios in tears of male and female rats

The influence of age on the FSC content in tears of male and female rats ($n=6$–13/gender/age) is shown in Fig. 3. At 0·6 months of age, FSC could not be detected in 13 out of 14 tear samples. By 1·3 months of age, though, tear FSC levels had increased substantially and in a gender-associated fashion: tear FSC content in males was significantly ($P<0·001$) higher than that of females. From 1·3 to 17 months of age, tear FSC levels were fairly constant in both males and females. During this post-pubertal time interval, tear FSC content was consistently geater in males than females.

These age- and gender-related variations in tear FSC levels were found irrespective of whether or not data were expressed in terms of total amounts, concentration or as a percentage of total tear protein. From 1·3 to 17 months of age, the total tear FSC amount in males was at least four-fold higher than that observed in females.

The relationship between FSC and IgA levels in tears as a function of age and gender is illustrated in Fig. 4. This comparison demonstrated that fluctuations in tear FSC and IgA during aging are not in parallel. The FSC/IgA ratio was highest in tears of 1·3-month-old rats, equalling 23·3±0·9% and 14·7±1·9% in males and females, respectively. This percentage progressively decreased as a function of age, such that by 17 months the FSC/IgA ratio in tears of males had undergone a significant ($P<0·001$), four-fold decline. The FSC/IgA ratio in tears of females also decreased during aging, but not to the same extent as that found in tears of males.

Impact of age and gender on the output of FSC by lacrimal tissue *in vitro*

The lacrimal gland is the principal tissue involved in the secretory immune system of the eye, and serves as the source of bound and free SC in tears (Brandtzaeg, 1985; Sullivan *et al.*, 1984a). To determine whether or not the FSC production by this gland varies as a function of age, lacrimal tissue segments from 0·6, 1·3, 3, 8 and 17-month-old male and female rats were cultured *in vitro* for 22 hr, and the incubation media then analysed for FSC content. As demonstrated in Fig. 5, lacrimal tissue from 0·6-month-old rats released very low quantities of FSC and this production was not gender-dependent. In contrast, the onset of puberty coincided with a striking, and sexually dimorphic, change in the FSC output by lacrimal glands. By 1·3 months of age, lacrimal tissue production of FSC increased by seven- and 15-fold amounts in glands obtained from females and males, respectively. In addition, the quantity of FSC released by tissues from male rats was significantly ($P<0·001$) higher than that secreted by glands from females. The output of FSC by lacrimal glands remained relatively stable in female rats beyond 1·3 months of age. In males, glandular FSC production continued to rise up to 3 months of age and then plateaued. In all post-pubertal age groups tested, glands from male rats released significantly more FSC than those of female rats.

The age-related fluctuations in FSC production by lacrimal tissue could be explained in part by variations in glandular weight. Thus, the amount of lacrimal tissue available in 0·6-month-old animals was approximately four-fold less than that of 1·3-month-old rats. However, as shown in Table 1, when lacrimal FSC output was corrected for tissue weight, significant increases in production were still found between glands from 0·6 to 1·3 month rats. Furthermore, gender-related differences in FSC production by lacrimal glands were observed irrespective of whether or not results were expressed in terms of total output or standardized to tissue weight.

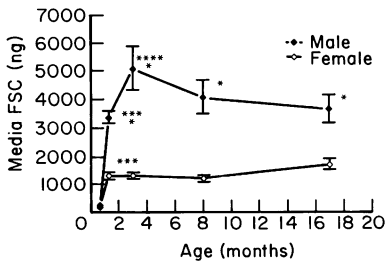

Figure 5. Age- and gender-related differences in FSC secretion by lacrimal glands incubated *in vitro*. Lacrimal glands were obtained from 0·6, 1·3, 3·0, 8·0 and 17-month-old male and female rats ($n=6$/gender/age). Tissues (0·5 gland/rat) were rinsed in saline, blotted on surgical gauze, halved and weighed. Tissue segments were placed in 20-ml glass vials containing 2 ml incubation medium, then gassed with 95% O_2–5% CO_2, capped and transferred to a shaking rack in a 37° water bath for 22 hr. Points represent the mean±SE. * Significantly ($P<0·005$) greater than value of age-matched female group; *** significantly ($P<0·005$) higher than value of previous gender-matched group; **** significantly ($P<0·05$) greater than value of previous gender-matched group.

Table 1. Effect of age and gender on FSC secretion by cultured lacrimal tissue

	Media FSC (ng)/lacrimal tissue weight (mg)	
Age (months)	Male	Female
0·6	20·2±4·6	16·5±1·8
1·3	74·6±8·8*†	33·5±3·6†
3·0	84·5±14·2‡	25·4±4·4
8·0	62·1±13·4‡	19·4±3·3
17·0	43·6±5·8‡	26·7±4·4

Lacrimal glands were obtained from 0·6, 1·3, 3·0, 8·0 and 17·0-month-old male (M) and female (F) rats ($n=6$ rats/gender/age) and tissues were cultured for 22 hr in incubation medium at 37° (see legend to Fig. 5). Tissue weights (mg) were as follows: 0·6 months, M=11·8±0·7 and F=10·5±1·1; 1·3 months, M=47·2±4·1 and F=39·3±2·7; 3·0 months, M=62·5±3·3 and F=56·8±7·4; 8·0 months, M=70·8±8·1 and F=69·5±7·4; 17 months, M=85·5±3·3 and F=71·0±10·3. Values represent the mean±SE of six samples/gender/age.

* Significantly ($P<0·005$) higher than value from female group.

† Significantly ($P<0·005$) greater than value from previous age group.

‡ Significantly ($P<0·05$) higher than value from female group.

Effect of age on the level of IgA, FSC and total protein in lacrimal gland cytosol of male rats

To evaluate whether age might influence the content of IgA, FSC or total protein in lacrimal gland cytosol, these parameters were examined in tissues from male rats ($n=6$–7/age) at 3 and 17 months of age. Male animals were selected for this comparison, because of the dramatic increase in tear IgA levels observed during this time period (Fig. 1). Prior to collection, lacrimal gland vasculature was perfused *in situ* with excess saline to remove residual blood, and then glands were processed to prepare cytosol. As demonstrated in Table 2, the lacrimal gland content of IgA rose approximately six-fold from 3 to 17 months of age. This increase in tissue IgA was found regardless of whether or not data were calculated in terms of total amounts, concentration or as a percentage of total protein. In addition,

Table 2. Impact of age on the level of FSC, IgA and total protein in lacrimal gland cytosol of male rats

Age (months)	FSC/protein (%)	IgA/protein (%)	Cytosol protein/ gland weight (%)
3·0	0·036±0·011	0·49±0·10	7·52±0·90
17·0	0·041±0·007	2·76±0·75*	7·55±0·32

Lacrimal glands were obtained from 3- and 17-month-old male rats (n=6–7/age) and utilized to prepare cytosol (refer to the Materials and Methods). Numbers represent the mean±SE of six to seven cytosol samples.

* Significantly ($P<0·05$) higher than IgA/protein ratio of 3-month-old rats.

Table 3. Influence of age on salivary IgA levels in male and female rats

	Saliva IgA (μg)/ml		% saliva IgA/protein	
Age (months)	Male	Female	Male	Female
3·0	85±8·5	91±18	0·91±0·12	0·99±0·15
17·0	118±20	93±17	1·34±0·21	1·47±0·33

Saliva was collected from 3- and 17-month-old male and female rats (n=6–7/gender/age). The salivary IgA/protein ratio was calculated by dividing the concentration of IgA by that of protein and multiplying the quotient by 100 (to obtain percentage). Numbers equal the mean±SE of six to seven samples/gender/age.

these results could not be explained by possible serum contamination in cytosol samples: the serum IgA/protein ratio in 17-month-old rats equalled 0·32±0·04%; this value was nine-fold less than that of cytosol. In contrast to the changes in IgA content, no effect of age was found on either tissue FSC levels or total protein amounts. In these studies, there was no significant difference between the lacrimal gland weights of 3- and 17-month-old rats.

Of interest in these experiments was the comparison between the IgA/protein and FSC/protein ratios in lacrimal tissue cytosol and tears of 17-month-old rats. As a percentage of total protein, IgA quantities increased from 2·76±0·75% in cytosol to 10·3±4·0% in tears. Similarly, the FSC/protein ratio rose from 0·041±0·007% in cytosol to 0·529±0·120% in tears. These differences indicate that IgA and FSC are secreted by lacrimal tissue into tears against an apparent concentration gradient.

In other studies we have also compared the amount of IgA and FSC in lacrimal gland cytosol obtained from 17-month-old male and female rats (n=6–7/group). Our results showed that tissues from male rats had three-fold higher levels of IgA and FSC, when expressed per protein, than did those of females. No significant difference was found between lacrimal gland weights of male and female rats.

Comparative influence of age on the IgA and FSC levels in saliva of male and female rats

To determine whether IgA and FSC levels in saliva might also vary as a function of age, salivary samples were collected from 3- and 17-month-old male and female rats (n=6–7/gender/age). As shown in Table 3, neither age nor gender appeared to influence the content of IgA in saliva. Moreover, no effect of age or gender was found on salivary FSC levels (Table 4). With regard to the FSC/IgA ratios in saliva, these decreased slightly from 3 to 17 months of age and were essentially identical in male and female rats (Table 4).

DISCUSSION

The present study demonstrates that aging has a marked impact on the secretory immune system of the eye. During a 3-week time span from before to after puberty, the level of IgA in tears of rats increased considerably, rising by factors of nine- and 13-fold in females and males, respectively. In addition, this developmental period coincided with parallel changes in the content of tear FSC: before puberty, FSC could not be detected in 93% of tear samples, whereas after pubertal maturation tear FSC levels had attained adult concentrations. Following these initial fluctuations, tear IgA content continued to increase in both sexes until adulthood (3 months of age), and then plateaued in females through mid-life to senescence. In contrast, tear IgA amounts in males appeared to stabilize until 8 months

Table 4. Effects of age and gender on the FSC content and the FSC/IgA ratio in rat saliva

	FSC (μg)/ml		%FSC/protein		%FSC/IgA	
Age (months)	Male	Female	Male	Female	Male	Female
3·0	4·1±0·4	3·6±0·5	0·043±0·004	0·040±0·004	4·93±0·60	4·55±0·80
17·0	4·1±0·8	3·0±0·5	0·046±0·006	0·047±0·009	3·51±0·18	3·08±0·31

Saliva was collected from 3- and 17-month-old male and female rats (n=6–7/age/gender). The FSC/protein ratio was determined by dividing the concentration of FSC (μg/ml) by that of protein (μg/ml) and multiplying the quotient by 100 (to obtain percentage). to calculate the FSC/IgA ratio, the concentrations of FSC were divided by those of IgA and the results were corrected for percentage. Values represent the mean±SE of six to seven samples/age/gender.

of age and then rose significantly up to 17 months of age. The magnitude of this latter rise was such that the tear IgA content in senescent males represented the highest level found in any age group. These age-related variations in tear IgA and FSC levels could not be accounted for changes in either the volume of, or total protein content in, tears.

The variations observed in the ocular secretory immune system during aging bear certain similarities to other mucosal sites. The minimal output of FSC by lacrimal tissue from 18-day-old rats, as evidenced by the very low FSC production by glands cultured *in vitro* and the absence of detectable tear SC *in vivo*, is analagous to the situation in the intestine. As reported by others, jejunal epithelial cells do not synthesize SC until rats are at least 2 weeks post-partum (Nagura, Nakane & Brown, 1978; Buts & Delacroix, 1985). Our finding that the maturational period spanning puberty is associated with a dramatic rise in the tear levels of IgA and FSC is also similar to the developmental response of other secretory immune sites: during puberty, IgA and SC content rise substantially in the mouth (Ebersole, Taubman & Smith, 1979; Kosaka, Asahine & Kobayashi, 1980) and intestine (Buts & Delacroix, 1985), respectively. Lastly, our observation that the tear FSC/IgA ratio declines progressively as a function of age correlates with changes found in this ratio for saliva (Kosaka *et al.*, 1980). The fact that FSC/IgA ratios are highest in secretions of young rats may reflect the earlier and faster development of SC-producing, as compared to IgA-producing, compartments in mucosal tissues (this study; Nagura *et al.*, 1978; Buts & Delacroix, 1985; Kosaka *et al.*, 1980).

The age-related fluctuations in the secretory immune system of the eye also show distinct disimilarities to other mucosal tissues. For example, the significant increase in tear IgA levels in male rats from adult to senescent ages does not appear to have a counterpart in another secretion. Analysis of different secretory immune sites in young and old rodents and humans has demonstrated either significant age-associated decreases in the concentrations of IgA in nasal (Alford, 1968) and intestinal (Lim *et al.*, 1981) secretions, or, as in saliva, no effect of age on total IgA content (this study; Smith *et al.*, 1983; Ebersole, Taubman & Smith, 1985). Moreover, researchers have reported a correlation between senescence and diminished hepatobiliary clearance of IgA antibodies (Schmucker *et al.*, 1985), lessened salivary IgA response to antigenic challenge (Smith *et al.*, 1983), and a marked reduction in the density of IgA-containing cells in the mesenteric lymph nodes (Haaijman & Hijmans, 1978). This last observation is of interest because the mesenteric lymph nodes are known to provide IgA-positive cells to many mucosal tissues, including the lacrimal gland (Montgomery *et al.*, 1983). Preliminary results from our laboratory, however, show that lacrimal glands from senescent rats harbor a fairly high number of IgA-containing cells (L. E. Hann, D. A. Sullivan and M. R. Allansmith, unpublished data). These age-related comparisons support the contention that the regulation of the ocular secretory immune system may be unlike that of other sites (Sullivan & Allansmith, 1985), and further, that the primary sources of IgA-positive cells in lacrimal gland may be from non-gut-associated lymphatic tissues (Brandtzaeg, 1985; Ebersole, Taubman & Smith, 1983).

There may be several explanations to account for the age-associated variations in tear IgA levels. One possibility is that the production of IgA by the lacrimal gland varies as a function of age. This may be likely in young rats, since the migration of IgA-producing cells into mucosal tissues apparently begins around the third week of life (Nagura *et al.*, 1978). Additional support for this hypothesis is our finding that the IgA content of lacrimal tissue increased significantly in male rats from 3 to 17 months of age. Whether this change was related to an elevated density of IgA-containing cells and/or an augmented IgA synthesis/cell remains to be established. A second possibility for tear IgA increases during aging is that the production of SC by the lacrimal gland rises in parallel, resulting in accelerated transport of tissue IgA into tears. This rationale follows from the known ability of SC to bind to, and facilitate, IgA movement into external secretions (Brandtzaeg, 1985; Solari & Kraehenbuhl, 1985). Our studies demonstrated that the secretion of FSC by the lacrimal gland, expressed either by the amount released into incubation media *in vitro* or into tears *in vivo*, increased dramatically from pre- to post-pubertal ages. This FSC output coincided in time with an elevation in tear IgA content. At later ages, though, tear IgA levels continued to rise, but not those of FSC. However, it is important to note that the total (i.e. free plus bound) amounts of SC synthesized and secreted by the lacrimal gland may also have risen after 1·3 months of age. Our SC radioimmunoassay detects primarily free, but not bound, SC (Sullivan & Wira, 1983b). Such an increase in total SC amounts would help explain the transfer of IgA from lacrimal tissue into tears against an apparent concentration gradient in senescent male rats. A third possibility for age-related fluctuations in tear IgA content is that they reflect alterations in the transfer of IgA from serum. However, this consideration is unlikely: the pattern of serum IgA concentrations during aging did not mirror the profile presented in tears, and IgA/protein ratios in serum were markedly below those recorded in tears. Furthermore, we have shown previously that tear IgA originates from the lacrimal gland, and not from serum (Sullivan & Allansmith, 1984).

A striking finding in the current studies was the pronounced sexual dimorphism in the ocular, but not oral, secretory immune system. In past studies, we have demonstrated that adult male rats (2–3 months old) express significantly higher levels of tear FSC (Sullivan *et al.*, 1984b) and IgA (Sullivan & Allansmith, 1985), an augmented synthesis and/or secretion of FSC by lacrimal tissue (Sullivan *et al.*, 1984a), and a greater density of IgA-positive cells in lacrimal glands (Sullivan *et al.*, 1986), when compared to female rats. These differences apparently are due to the influence of androgenic hormones (Sullivan *et al.*, 1984a,b; Sullivan & Allansmith, 1985), which also regulate the morphology, histochemistry, biochemistry and genetics of the lacrimal gland (Cavallero, 1967; Hahn, 1968; Paulini, Beneke & Kaulka, 1972; Shaw, Held & Hastie, 1983). The present experiments confirm and extend these previous results. However, our findings also raise questions as to whether or not the underlying mechanism of gender-related differences in ocular mucosal immunity can be attributed solely to androgenic action. Thus, a significant and increasing disparity exists between tear IgA levels of male and female rats from adulthood to senescence; yet, during this time interval, serum testosterone concentrations (Steiner *et al.*, 1984; Swerdloff & Heber, 1982) and the quantity of androgen receptors in peripheral tissues (Roth, 1979) undergo a marked decrease. One possible reason for our observation is that the hypothalamic–pituitary axis, and not testosterone, is actually responsible for gender-associated differences in the secretory immune system of the eye. In support of

this hypothesis, recently we have found that the testosterone regulation of tear SC and IgA levels is completely blocked if rats are hypophysectomized (Sullivan & Allansmith, 1987). Furthermore, pituitary hormones, whose release is gender-dependent (Ramaley, 1979; Bhatnagar, 1983), are known to exert significant influence on the lacrimal gland (Cavallero *et al.*, 1960; Martinazzi & Baroni, 1963; Ebling *et al.*, 1975; Jahn *et al.*, 1982).

In summary, our findings indicate that the secretory immune system of the eye is either conserved or enhanced as a function of age. In addition, our results demonstrate that the extent of these age-associated variations is dependent upon gender.

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