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Source of IgA in tears of rats

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Summary. The purpose of the present study was to determine whether IgA in rat tears originates from serum and/or local synthesis. To examine the first possibility, we compared the IgA levels in serum and tears of rats with a portacaval anastomosis. This operation induced a chronic and progressive elevation of polymeric IgA concentrations in serum. By 8 weeks after surgery, serum IgA levels in 'portacaval' rats were 20-fold higher than those of sham-operated or intact controls. In contrast, IgA levels in tears of 'portacaval' rats did not increase after surgery, despite the availability of free secretory component (SC) in tears. The lack of correlation between tear and serum IgA concentrations resulted in a significant decrease in the tear IgA/serum IgA ratio after anastomosis surgery.

To assess whether tear IgA might be derived from local synthesis, we cultured various ocular tissues from male rats in the presence or absence of cycloheximide, an inhibitor of protein synthesis. Incubation of exorbital (lacrimal) glands resulted in the accumulation of substantial quantities of IgA in the culture medium. This accumulation was significantly reduced by the presence of cycloheximide. In contrast, cycloheximide had no effect on the amounts of IgA in cultures of 'lids', Harder's glands and globes.

These results indicate that IgA in rat tears originates from local synthesis and not from serum transfer. Furthermore, our findings suggest that the rat exorbital gland is responsible for the synthesis and secretion of tear IgA.

Abbreviations: BSA, bovine serum albumin; BW, body weight; IgA, immunoglobulin A; LW, liver weight; PB8, 0·1 M sodium phosphate buffer, pH 8·0; RT, room temperature; SC, secretory component; sIgA, secretory IgA; TKM, 50 mM Trizma, 25 mM KCl, 5 mM $MgCl_2$, pH 7·5.

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INTRODUCTION

Secretory IgA (sIgA), the predominant antibody in mucosal secretions, is thought to act as an immunological barrier to the adherence, colonization and entry of invasive microbial agents (Ganguly & Waldman, 1980). The source of this IgA is, however, controversial: depending upon the site, the origin of IgA may be attributed either to local synthesis and/or serum deposition. Local production of IgA occurs in plasma cells located in the mucosal lamina propria, which underlies the luminal surface epithelium (Lamm, 1976). Serum polymeric IgA, though, may also gain access to mucosal tissues and secretions, such as saliva (Montgomery *et al.*, 1977), colostrum (Halsey *et al.*, 1982; Sheldrake *et al.*, 1984), bile (Fisher *et al.*, 1979), urine (Jackson, Lemaitre-Coelho & Vaerman, 1979) and bronchial (Lemaitre-Coelho *et al.*, 1982) and uterine fluids (Sullivan & Wira, 1983a, 1984). This serum IgA may originate in large part from intestinal plasma cells (Vaerman *et al.*, 1973), and has been suggested to represent a major input of IgA into external secretions of the rat (Lemaitre-Coelho, Jackson & Vaerman, 1978b).

As concerns the ocular secretory immune system,

the proportional contributions of local synthesis versus serum transfer to the concentration of IgA in tears has not been determined. A high density of IgA-containing cells has been observed in the lacrimal glands of several species (Allansmith *et al.*, 1976; Franklin, Prendergast & Silverstein, 1979; Gudmundsson *et al.*, 1984), but the presence of IgA in plasma cells does not necessarily indicate lymphocytic secretion of the immunoglobulin (Conley & Koopman, 1982). In fact, researchers have speculated that the tear IgA originates primarily from serum (Hall, Hopkins & Orlans, 1977) and studies on lactating rats support this possibility (Kleinman *et al.*, 1983). Moreover, experiments involving topical antigenic challenge of the eye have met with variable success in inducing local antibody appearance (Murray, Charbonnet & MacDonald, 1973; Hall & Pribnow, 1981; Montgomery *et al.*, 1983). Thus, it has been proposed that serum, as well as tear, antibodies correlate equally well with ocular resistance to infectious agents (Malaty *et al.*, 1981).

The purpose of the present study was to determine whether tear IgA is derived from serum transfer and/or local production. Towards this end, we have measured tear IgA concentrations in rats with chronically elevated levels of serum polymeric IgA. This immune condition was induced by the surgical formation of a portacaval anastomosis (Vaerman *et al.*, 1981) and offsets the rapid clearance problems encountered with the administration of radiolabelled polymeric IgA to normal rats (Fisher *et al.*, 1979). We have also investigated whether ocular tissues produce IgA *in vitro*.

MATERIALS AND METHODS

Animals and surgical procedures

Adult male Sprague-Dawley rats were maintained in constant temperature rooms with fixed light/dark intervals of 12 h length. Animals (11 weeks old; $n = 7$) used for the tissue incubation studies were purchased from Charles River Breeding Laboratories (Wilmington, MA). Rats utilized in the portacaval anastomosis experiment were obtained from Zivic-Miller Laboratories, Inc. (Allison Park, PA) which also performed the surgical procedures. Animals underwent portacaval anastomosis (Eck fistula) operations ($n = 10$) or sham-surgery ($n = 9$) at 5 weeks of age and were delivered to our laboratory with a group of intact, age-matched rats ($n = 9$) 1 week later. Criteria used to verify succcess of anastomotic surgery included body and liver weight analysis (Lee *et al.*, 1974), as well as the monitoring of serum IgA levels (Vaerman *et al.*, 1981).

General methods

Tears were collected from ether-anaesthetized rats and diluted in sodium phosphate buffer (0·1 M pH 7·0), as previously described (Sullivan, Bloch & Allansmith, 1984b). Blood was obtained from the tail, then allowed to clot at room temperature (RT). Samples were centrifuged at 10,000 ***g*** for 4 min and supernatants were stored at −20°.

Prior to incubation of exorbital glands *in vitro*, ocular tissue vasculature was perfused *in situ* to remove residual blood. This procedure involved cannulation of the descending aorta immediately below the diaphragm with a 19-gauge needle, which was attached by tubing to a raised saline reservoir. Perfusion was directed in a retrograde fashion towards the heart. Under these conditions, the aortic valve is shut and flow traverses the ascending aortic branch. The right atrium was severed to permit venous escape of fluid. After 50–100 ml saline had been perfused, exorbital glands were removed and incubated in the presence or absence of cycloheximide (Sigma, St Louis, MO; 100 μg/ml), according to previously reported techniques (Sullivan, Bloch & Allansmith, 1984a). In other experiments, exorbital glands, Harder's glands, globes and lids with adjacent tissues (conjunctiva, tarsal plates and muscle; termed 'lid') were removed without prior perfusion *in situ* and cultured as described (Sullivan *et al.*, 1984a). Following incubation, culture media were centrifuged at 10,000 ***g*** for 4 min and the supernatants were frozen at −20°.

The Student's *t*-test was utilized to determine significance of the data.

Chromatography

To characterize the molecular form of IgA (i.e. polymeric *vs* monomeric) in 'portacaval' rat serum, a 540 μl sample of pooled serum was applied to a 0·9 × 60 cm column of Bio-Gel A1.5 (Bio-Rad, Richmond, CA). Filtration was performed at 3° with a 0·1 M sodium phosphate buffer, pH 7·0, containing bovine serum albumin (0·5 mg/ml; Calbiochem-Behring Corp., San Diego, CA). Eluates (0·9 ml/tube) were collected at a rate of approximately three drops/-minute and analysed by radioimmunoassay (RIA) for both IgA and SC (see below). To standardize the

column, elution profiles of polymeric and monomeric IgA and SC from rat saliva, tears and/or serum, as well as ^{125}I-SC, were determined.

Antisera and antigens

Goat anti-rabbit IgG, rabbit anti-goat IgG and goat anti-rat IgA, as well as rat IgG and rat reference serum, were purchased from Miles Laboratories (Elkhart, IN). Anti-rat secretory component (SC) antisera (two sources: one was raised against rat colostral SC, given by Dr S. Michalek, Birmingham, AL, and reactive with sIgA in immunoelectrophoresis; and the other was raised against rat bile SC, given by Dr C. Wira, Hanover, NH and used in the SC RIA) and purified rat SC (donated by Dr Wira) were prepared by Dr Brian Underdown (Toronto, Canada). Rat IgA was isolated by affinity chromatography. Briefly, rat serum (10 ml) was diluted two-fold with 0·1 M sodium phosphate buffer, pH 8·0 (PB8) and run slowly over a 3·1 ml affinity column, which consisted of purified mouse monoclonal antibodies to rat IgA (D. A. Sullivan & C. R. Wira, unpublished information) bound to CNBr-activated Sepharose-4B (Pharmacia Fine Chemicals, Piscataway, NJ). Following exhaustive washes with PB8 and PB8/0·5 M NaCl buffers to remove unbound protein, rat IgA was eluted with 0·1 M glycine/HCl buffer, pH 2·5. To neutralize the acid, eluates were collected in tubes containing aliquots of 1 M Trizma.

Measurement of IgA and SC

Free SC in tears, gel filtration eluates and culture media were measured by a previously characterized RIA (Sullivan & Wira, 1983b). Rat IgA levels were also measured by RIA, which was developed by using the double antibody precipitation technique. Assay components included rat reference serum, which contained 243 μg IgA/ml, as a standard, iodinated rat IgA, goat anti-rat IgA as the first antibody and rabbit anti-goat IgG as the second antibody. Affinity-purified rat IgA, after five-fold concentration, was iodinated with ^{125}NaI (New England Nuclear, Boston, MA) by using IODO-GEN (Pierce Biochemical, Rockford, IL; Markwell & Fox, 1978) as previously described (Sullivan & Wira, 1983a). First antibody concentrations were utilized which bound approximately 90% of the radiolabelled IgA. Replacement of the anti-IgA with normal goat serum resulted in background (assay blank) counts which equalled less than 1% of the total radioactivity. Second antibodies were added in amounts capable of binding all first antibody, as demonstrated by serial dilutions of the rabbit anti-goat IgG. Excess unlabelled rat IgG was added to block cross-reacting antibodies in the second antibody preparation to rat heavy and/or light chains.

To run each RIA, IgA standards or samples were added in 10–20 μl aliquots to 1·5 ml polypropylene tubes (Sarstedt, Princeton, NJ). The diluent utilized for the standards and samples was either 0·1 M sodium phosphate, pH 7·0, containing 1 mg BSA/ml, or culture medium (RPMI 1640, Gibco, Lawrence, MA; 10% fetal bovine serum with glutamine, Gibco; and 25 μg garamycin/ml, Schering Corporation). The vehicle used for ^{125}I-IgA and first and second antibodies was BSA/TKM (1 mg/ml; TKM: 50 mM Trizma, Sigma, 25 mM KCl, 5 mM $MgCl_2$, pH 7·5). Following standard or sample addition, 30 μl ^{125}I-IgA and 20 μl goat anti-rat IgA (0·04%) were transferred to the tubes. After vortexing, then incubating the assay tube contents for 1 hr at RT, 30 μl of a fixed rabbit anti-goat IgG dilution (1/40) were added. Tubes were vortexed, incubated for 1 hr at RT and then centrifuged at 10,000 *g* for 4 min. Supernatants were aspirated and pellets were washed with 800 μl of a 0·15 M NaCl mixture, containing 0·05% Tween 20 (Fisher Scientific, Medford, MA) and 1 mg carboxymethylcellulose/ml (Microgranular CM52, preswollen, Whatman, Kent, U.K.); this wash solution was a critical factor in obviating background variations in the RIA. Tubes were again centrifuged, wash fluid was removed and pellet radioactivity was counted in a Beckman scintillation spectrometer. The incubation intervals used in this assay were determined by time course studies, and precipitation was not enhanced by the extension of these incubations.

Characterization of the IgA RIA

To determine the IgA levels in tears, serum, tissue incubation media and chromatographic eluates, standard curves were included with each assay and analysed by log logit transformation. The range of the standard curve extended from 4·9 to 486 ng IgA (0·02 to 2 μl reference serum). Analysis of 17 different curves resulted in the following assay characteristics: slope $= -0{\cdot}367 \pm 0{\cdot}010$ (mean ± SE); 50% displacement of ^{125}I-IgA $= 30{\cdot}7 \pm 1{\cdot}9$ ng IgA; correlation coefficient $= 1{\cdot}0 \pm 0{\cdot}0$. Within assay variability of 16 samples was $4{\cdot}0 \pm 0{\cdot}8\%$; between assay variation of 12 samples was $6{\cdot}2 \pm 1{\cdot}2\%$. The assay sensitivity, defined as the lowest amount of IgA measurable and equal to the quantity two standard deviations above the zero dose response, was 3·3 ng IgA.

In order to evaluate whether samples interfered with the IgA RIA, measurements were first verified by serial dilution of, and IgA standard addition to, the various samples. Sample dilution yielded IgA values which were essentially parallel to the standard curve. Moreover, when known amounts of IgA were added to samples, no significant interference in measurement of the standard was evident.

RESULTS

Effect of chronic elevation of serum IgA levels on the IgA concentration in tears

The surgical formation of a portacaval anastomosis resulted in a marked elevation in the IgA levels in rat serum. As shown in Fig. 1, serum IgA concentrations in 'portacaval' rats were significantly greater than those of sham-operated or intact rats by 18 days after surgery. These levels increased progressively over the time course of this experiment, such that by 8 weeks after surgery, IgA concentrations in 'portacaval' serum were 20-fold higher than those measured in control serum. No significant variations were observed between the serum IgA amounts in sham-operated or intact rats throughout this study.

Analysis of 'portacaval' rat serum (collected from rats 8 weeks after surgery) by gel filtration revealed that the increase in IgA was primarily of the polymeric form and that free SC could not be detected. Moreover, immunoelectrophoretic examination of 'portacaval' serum IgA showed a single precipitin band against goat anti-rat IgA, but no reaction with rabbit anti-rat SC. These results indicate that the portacaval anastomosis operation induced a chronic elevation in serum polymeric, but not secretory, IgA.

The IgA levels in tears of 'portacaval' rats did not increase after surgery. Instead, tear IgA concentrations in these animals were significantly less than those measured in sham or intact controls during the time couse of this experiment (Fig. 2). This comparative decrease in tear IgA amounts was not due to variations in tear volume or an absence of free SC. Tear volumes were not significantly different among the intact, sham-operated and 'portacaval' groups (Table 1). Furthermore, free SC was present in tears of 'portacaval' rats, though in lesser amounts than found in control rats. The free SC/IgA ratio in tears was similar in all three experimental groups (Table 1).

The lack of correlation between serum and tear IgA

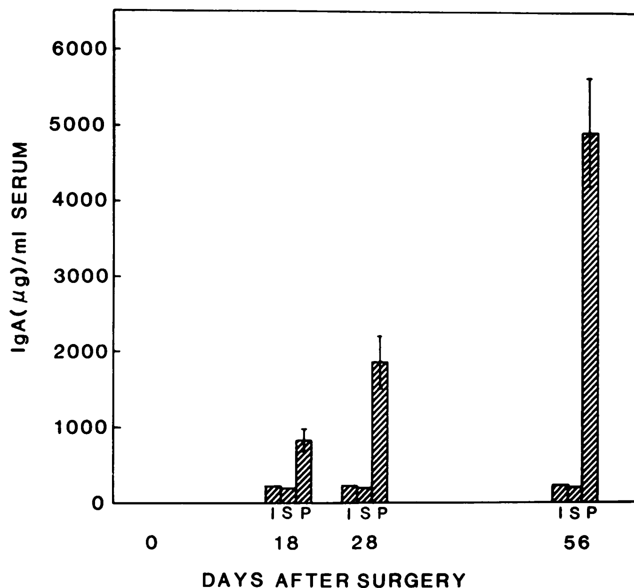

Figure 1. Influence of portacaval anastomosis surgery on IgA levels in rat serum. Serum was collected from intact (I, $n = 9$–10), sham-operated (S, $n = 8$–9) and operated (portacaval anastomosis, P, $n = 8$–9) rats on days 18, 28, and 56 after surgery. Bars represent the mean ± SE. Serum IgA concentrations in 'portacaval' rats were significantly ($P < 0{\cdot}001$) greater than IgA levels in sham-operated rats. Immunoglobulin A concentrations in serum from intact and sham-operated rats were not significantly different.

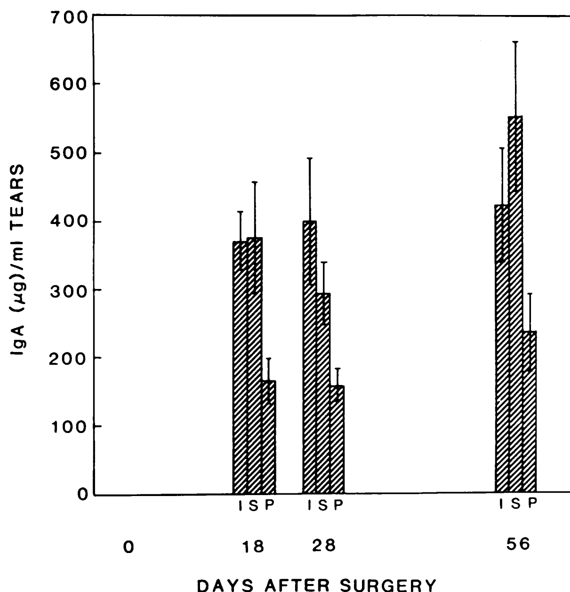

Figure 2. Tear IgA concentrations in rats with chronically elevated levels of serum polymeric IgA. Tears were obtained from intact (I, $n=9$–10), sham-operated (S, $n=8$–9) and operated (portacaval anastomosis, P, $n=8$–10) rats on various days after surgery. The anastomosis surgery induced a progressive increase in serum IgA concentrations (see Fig. 1, text). Bars equal the mean ± SE. Tear IgA concentrations in 'portacaval' rats were significantly ($P<0{\cdot}05$) less than those of sham-operated controls. No significant differences were found between tear IgA levels in intact and sham-operated rats.

Table 1. Effect of portacaval anastomosis on tear volumes, free SC and IgA levels and free SC/IgA ratios in tears of male rats

Group	Number	Tears (μl)	Free SC (ng)	IgA (ng)	Free SC (ng)/IgA (ng)
Intact	9	4·8±0·9	344±54	1590±171	0·214±0·023
Sham	8	4·9±1·0	401±56	2014±208	0·204±0·028
Portacaval	8	5·8±0·9	211±43*	1074±109†	0·213±0·053

Samples were obtained from age-matched rats which had undergone portacaval anastomosis surgery (8), sham surgery (8) or no surgery (intact; 9) 56 days prior to tear collection. The free SC and IgA numbers refer to the absolute amounts recovered in the tear samples. No significant differences were found between the above values obtained from intact and sham-operated groups.

* Significantly ($P<0{\cdot}01$) less than sham-operated control.

† Significantly ($P<0{\cdot}001$) less than sham-operated control.

levels is shown in Fig. 3. In 'portacaval' rats, the tear IgA/serum IgA ratios were significantly below those calculated for controls and declined four-fold during the experimental period. The tear IgA/serum IgA ratios of sham-operated and intact rats were not significantly different.

The effects of portacaval anastomosis on body and organ weight are listed in Table 2. Body weight (BW), liver weight (LW) and LW/BW ratios of 'portacaval' rats were significantly decreased compared to controls. Rats that had undergone sham surgery also had reduced body and liver weights, but their mean LW/BW ratio was identical to that of intact controls. With regards to exorbital glands, these tissues weighed

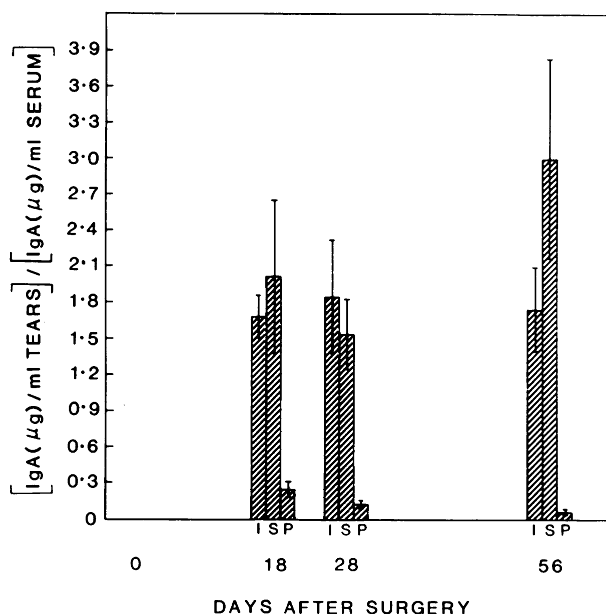

Figure 3. Comparison between tear IgA/serum IgA ratios in rats with or without portacaval anastomosis surgery. Sample collection procedures and experimental group designations are described in the legends to Figs 1 and 2. Bars represent the mean ± SE. The tear IgA/serum IgA ratios of rats with a portacaval anastomosis were significantly ($P<0{\cdot}005$) less than ratios of sham-operated controls and underwent a significant ($P<0{\cdot}01$) decline from days 18 to 28 and 28 to 56 after surgery. The tear IgA/serum IgA ratios in sham-operated and intact animals were not significantly different.

less in 'portacaval' animals, but the exorbital gland/BW ratios were similar to those of intact rats (Table 2).

Production of IgA by ocular tissues *in vitro*

In order to determine whether tear IgA might be derived from local synthesis, saline-perfused exorbital glands from male rats were incubated for 20 hr in the presence or absence of cycloheximide (100 μg/ml medium), an inhibitor of protein synthesis. Glandular incubation resulted in the accumulation of substantial quantities of IgA in the culture medium (Table 3). When cycloheximide was added to the incubation medium, IgA production by exorbital glands was reduced by 43 ± 3% (mean ± SE).

The inhibitory effect of cycloheximide on the IgA accumulation in exorbital gland cultures was also found in additional studies, which involved overnight incubation of non-perfused tissues ($n=6$ rats/experi-

Table 2. Effect of portacaval anastomosis on rat organ weight, body weight and organ weight/body weight ratios

Group	Number	BW (g)	Liver wt (g)	Liver wt (g)/BW (g)	Exorbital gland wt (mg)	Exor (mg)/BW (g)
Intact	9	536 ± 7	19·9 ± 0·6	3·71 ± 0·09	323 ± 18	0·604 ± 0·035
Sham	8	485 ± 16*	18·0 ± 0·7†	3·71 ± 0·07	289 ± 14	0·595 ± 0·017
Portacaval	8	293 ± 26‡	7·5 ± 0·9‡	2·50 ± 0·11‡	196 ± 19‡	0·668 ± 0·027§

Age-matched rats had portacaval, sham or no surgery 8 weeks prior to measurements. Exorbital gland weight equals total of both left and right glands.

* Significantly ($P<0{\cdot}005$) less than body weight of intact control.

† Significantly ($P<0{\cdot}05$) less than liver weight of intact control.

‡ Significantly ($P<0{\cdot}001$) less than appropriate sham control.

§ Significantly ($P<0{\cdot}05$) greater than sham, but not intact, control.

Table 3. Production of IgA by the exorbital gland *in vitro*

Sample	Number	Weight (mg) of exorbital tissue	Media IgA (μg)	Media IgA (μg)/tissue wt (mg)
Cycloheximide$^-$	7	104±6	34·3±6·1	0·333±0·057
Cycloheximide$^+$	7	112±10	20·6±3·7*	0·189±0·033*

Left and right exorbital glands were removed from rats ($n=7$) after saline perfusion of vasculature to remove residual blood. Tissues were rinsed in saline, blotted on surgical gauze, quartered and weighed. Tissue segments were divided and combined in such a way that approximately half of each tissue was exposed to cycloheximide. Tissues were placed in 20 ml glass vials containing 2 ml of prewarmed incubation medium in the presence (+) or absence (−) of cycloheximide (100 μg/ml). Vials were gassed with 95% O_2–5% CO_2, capped and transferred to a shaking rack in a 37° water bath for 20 hr.

* Significantly ($P<0·05$) less than value obtained from samples not exposed to cycloheximide.

ment). In two separate experiments, IgA production (standardized to weight of tissues) was decreased by 32±5 and 45±8% by cycloheximide presence. In contrast, cycloheximide had no effect on the accumulation of IgA in media of 'lid', globe, or Harder's gland cultures.

During these studies, we also examined the relationship between IgA and SC production by exorbital glands *in vitro*. In different experiments ($n=6$ rats/study), the free SC/IgA ratio in culture media following overnight gland incubation equalled 0·20±0·05 and 0·19±0·02. These values are analagous to those found *in vivo* in tears.

DISCUSSION

The results of the present study indicate that IgA in rat tears originates from local synthesis and not from serum transfer. This conclusion is based upon two principle observations: (i) the surgically-induced, chronic elevation of serum polymeric IgA levels did not elicit a reciprocal increase in the tear IgA concentrations; (ii) incubation of exorbital glands *in vitro* led to the accumulation of substantial quantities of IgA in the culture medium. This IgA production was signficantly reduced by the presence of cycloheximide.

The chronic elevation in serum polymeric IgA levels was achieved by the surgical creation of a portacaval anastomosis, as originally reported by Vaerman *et al.* (1981). This operation results in a significant decline in body weight, liver weight and LW/BW ratio (Lee *et al.*, 1974) and causes a marked alteration in both hepatocyte histology (Lee *et al.*, 1974; Ronchetti *et al.*, 1983) and function (Lee *et al.*, 1974; Lauterburg *et al.*, 1976). This decrease in liver mass, coupled with the hepatocyte impairment, most likely accounts for the progressive increase in serum IgA concentrations. Typically, the rat liver rapidly clears polymeric IgA from the circulation (Fisher *et al.*, 1979) and experimental attempts to saturate this process with large doses of IgA have proven unsuccessful (Lemaitre-Coelho *et al.*, 1981). Consequently, studies designed to examine the possible movement of exogenously-administered IgA from serum into mucosal secretions are difficult to perform because of the rapid hepatic clearance. One method that has been utilized to compromise this liver function involves bile duct ligation (Dahlgren *et al.*, 1981; Lemaitre-Coelho *et al.*, 1982). However, this procedure results in heightened serum concentrations of sIgA and free SC (Lemaitre-Coelho, Jackson & Vaerman, 1978a), which binds to polymeric IgA (Fisher *et al.*, 1979), and serum sIgA is not transported by mucosal tissues into external secretions (Fisher *et al.*, 1979; Lemaitre-Coelho *et al.*, 1981). Therefore, the 'portacaval' rat, in which polymeric, but not secretory, IgA levels are increased in serum, appears to be an ideal model to assess the contribution of circulating IgA to mucosal sites.

Our analysis of tear IgA concentrations in 'portacaval' rats indicated that serum IgA was not transferred into tears. This lack of transport was especially evident by 8 weeks after surgery, when serum IgA levels had increased 20-fold, but no correlative rise in tear IgA amounts had occurred. One possible explanation for this finding is that serum polymeric IgA did not gain entrance to the exorbital gland, which produces tear

SC (Sullivan *et al*, 1984a). As demonstrated in the uterus (Sullivan *et al*., 1983; Sullivan & Wira, 1984) and liver (Fisher *et al*., 1979; Lemaitre-Coelho *et al*., 1981) of rats, the tissue availability of SC and polymeric IgA from serum results in the movement of IgA into external secretions. Since free SC was present in tears of 'portacaval' rats, and presumably in glandular tissue, we suggest that molecular weight restrictions may have prevented access of serum polmeric IgA to tissue SC. The absence of correlation between tear and serum IgA is consistent with the experimental findings of others (Mestecky *et al*., 1978; Burns, Ebersole & Allansmith, 1982; Montgomery *et al*., 1983), who observed that IgA antibody levels in these compartments were not interdependent.

An intriguing finding in the present study was that IgA and SC levels in tears of 'portacaval' rats were lower than those of sham-operated or intact rats. This difference might reflect a relative decrease in protein secretion due to the reduced weight of exorbital glands in animals with portacaval anastomoses. The similarity between free SC/IgA ratios in all three groups would support this hypothesis. However, an alternative explanation for the lower tear IgA levels in 'portacaval' animals is that this operation may have interfered with the androgen modulation of the ocular secretory immune system (Sullivan *et al*., 1984a, 1984b). Portacaval anastomosis surgery suppresses the hypothalamic-pituitary-gonadal axis and results in a pronounced testicular atrophy and a marked decrease in circulating testosterone levels (Lee *et al*., 1974; Milligan & Sarna, 1981). Since androgens appear to regulate the concentrations of SC (Sullivan *et al*., 1984b) and IgA (Sullivan & Allansmith, 1984) in tears, a diminished androgen influence in 'portacaval' rats might have led to decreased tear IgA levels.

The cycloheximide-sensitive production of IgA by the exorbital gland, and not by the 'lid', Harder's gland or globe, suggests that this tissue plays the primary role in the ocular secretory immune system. Consistent with this hypothesis are the findings that the exorbital gland, and not other ocular tissues, has a high density of IgA-containing cells (Gudmundsson *et al*., 1984) and produces large amounts of SC (Sullivan *et al*., 1984a). Moreover, the free SC/IgA ratio in culture media of exorbital glands was essentially equal to that in tears. These results indicate that the rat exorbital gland, like the human lacrimal gland (Allansmith *et al*., 1976; Allansmith & Gillette, 1980), is most likely responsible for the synthesis and secretion of tear IgA.

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