Immunology 1979 **38** 33

The neonatally thymectomized rat: a model for compensatory IgM antibody formation in exocrine secretions

J. L. EBERSOLE, M. A. TAUBMAN & D. J. SMITH *Department of Immunology, Forsyth Dental Center, Boston, Massachusetts, U.S.A.*

Accepted for publication 13 March 1979

Summary. The effect of T-cell deprivation on IgM antibodies in saliva was studied in rats. IgM was never detected in salivas obtained from normal or sham-thymectomized rats, but was detected in saliva samples from 8/15 (53·3%) rats that had undergone neonatal thymectomy. All (6/6) neonatally thymectomized rats exhibited an IgM antibody response to DNP in salivary secretions after local immunization with a T-dependent antigen (DNPBGG), while no IgA anti-DNP activity was detected in saliva from these animals. IgM antibodies were detected in saliva from 5/7 thymectomized rats following local injection with a T-independent antigen (DNP-Lys-Ficoll). This was accompanied by detectable but substantially reduced levels of secretory IgA antibody in saliva from 7/7 rats. The results suggested that absent or decreased salivary IgA responses accompanying T-cell deprivation in the rats are compensated for by secretion of IgM antibodies into the saliva. The neonatally thymectomized rat may thus provide a model for the study of synthesis, secretion and protective potential of exocrine IgM antibodies.

INTRODUCTION

The predominant immunoglobulin in exocrine secretions from normal individuals is immunoglobulin A (IgA) (Tomasi & Bienenstock, 1968). This IgA has been shown to be synthesized primarily by immunocytes that reside in proximity to glandular epithelium (Tourville, Adler, Bienenstock & Tomasi, 1969). The full functional capabilities of IgA in the external secretions are not completely understood; this Ig class, however, appears to afford a primary protective capacity at the mucous surfaces of the body (Waldman & Ganguly, 1974). Early studies examining Ig levels in external secretions showed that a proportion (1 in 500 to 1 in 700) of apparently healthy humans demonstrate a selective deficiency in both serum and secretory IgA (Rockey, Hanson, Heremans & Kunkel, 1964). The intestinal mucosae of these individuals are enriched in IgM-containing cells, unlike the mucosae of normal individuals in which IgA-containing cells are most abundant (Crabbé, Carbonera & Heremans, 1965). This enrichment of IgM cells at the mucous membranes has also been reflected in studies of exocrine secretions where locally synthesized IgM has been shown to be the predominant Ig in saliva (Brandtzaeg, Fjellanger & Gjeruldsen, 1968; Brandtzaeg, 1971; Savilhati, 1973). Ogra and his colleagues (Ogra, Coppola, MacGillivray & Dzierba, 1974) have also demonstrated that local immunization of IgA-deficient individuals results in a 'compensatory' antibody response composed of both IgM and IgG. Whether these IgM and IgG antibodies replace the primary protective features of IgA remains unclear. Recent results from our laboratory have shown that thymec-

Correspondence: Dr Jeffrey L. Ebersole, Department of Immunology, Forsyth Dental Center, 140 Fenway, Boston, Massachusetts 02115, U.S.A.

0019-2805/79/0900-0033$02.00

tomy of neonatal rats decreased IgA levels in saliva and serum by nearly 80 and 70%, respectively (Ebersole, Taubman & Smith, 1979). This system has potential as a model to examine the salivary immune response in conditions of IgA deficiency. The present study was initiated to determine whether IgM could be detected in saliva following neonatal thymectomy. The results suggest that under the condition of T-cell deprivation with attendent diminished IgA synthesis, IgM comprises a portion of the immune response in secretions. Therefore, it is suggested that the neonatally thymectomized rat can constitute a model to study the synthesis, secretion and protective potential of compensatory exocrine IgM antibodies.

MATERIALS AND METHODS

Thymectomy

Neonatal thymectomy (Tx) was performed on Sprague–Dawley (CD strain) rats within 24 h of birth using vacuum aspiration (Miller, 1960). Groups of rats were also sham-thymectomized (STx) by subjecting them to the entire surgical procedure without removal of the thymus. A normal (N) group of rats was age matched to the surgically treated animals. Total and differential white blood cell counts of 35 day old rats together with histological and stereomicroscopic mediastinal examinations immediately after death were used to ensure the absence of thymic tissue in experimental animals. Immunofluorescent examination of lymphocytes isolated from various tissues of rats that were thymectomized as neonates showed greatly diminished T-cell numbers in the spleen (7%); peripheral blood (13·5%) and bone marrow (0%) when compared to the percentages of T cells in normal rats (spleen, 55%; peripheral blood, 63·5%; bone marrow, 9·5%).

Immunoglobulin levels

Immunoglobulin levels in saliva and serum were quantified by a single radial immunodiffusion procedure (Mancini, Carbonera & Heremens, 1965). Rabbit anti-rat μ chain globulin was incorporated into 1% buffered agarose. Equal volumes of the samples were place in wells and allowed to diffuse for 48 h at room temperature in a humidified chamber. To increase the sensitivity of the assay for IgM, anti-μ plates were subsequently washed in phosphate-buffered saline (PBS) for 16–18 h and overlayed with filter paper (No. 2 Whatman) soaked in a solution of goat anti-rabbit γ globulin serum (Miles Biochemicals, Elkhart, IN) for 16–18 h according to Brandtzaeg & Tolo (1977). The plates were then washed, dried, stained and the rings measured as described previously (Ebersole *et al.*, 1979). A serum with known Ig concentrations (Ebersole *et al.*, 1979) was appropriately diluted and used to generate a standard curve. The plates as prepared could detect immunoglobulin concentrations greater than, or equal to, IgM: 1·6 μg/ml, IgG: 3 μg/ml, and IgA: 8 μg/ml. The IgM concentrations obtained using an RID procedure could be artificially high if the whole salivas contained quantities of monomeric IgM. Therefore, the molecular size of salivary IgM was estimated by pooling saliva from normal or neonatally thymectomized rats and subjecting each pool to gel chromatography on a column of Sephadex G-200. The eluted fractions of the saliva pools gave similar patterns of absorbance at 230 nm. Pooled fractions were subsequently analysed by the RID procedure. IgM was detected only in the void volume region of gel filtered whole saliva from the neonatally thymectomized rats, consistent with the high molecular weight of IgM. No IgM was detected in any fraction from the normal rats by this assay.

Antigens

A T-independent antigen, ε-2,4-dinitrophenyl lysyl Ficoll (DNP-Lys-Ficoll) (McMaster, Owens & Vannier, 1977) containing 38 moles of DNP-Lys per 40,000 mol. wt units of carbohydrate was prepared according to Sharon, McMaster, Kask, Owens & Paul (1975). The concentration of ε-DNP-lysine groups was determined spectrophotometrically at 360 nm (Eisen, Belman & Carsten, 1953) and the concentration of Ficoll in the conjugate was determined by the phenol–sulphuric acid reaction (Dubois, Gilles, Hamilton, Robers & Smith, 1956). A T-dependent antigen, ε-2, 4-dinitrophenyl bovine gamma globulin (DNPBGG), containing 16 DNP groups per molecule of BGG was prepared according to Eisen *et al.* (1953). The degree of substitution was obtained by dry weight determination of BGG; and DNP was analysed by spectrophotometric measurements as described. The conjugates were prepared so as to administer equal molar amounts of DNP to the immunized rats (38–40 DNP groups/400,00 mol. wt units of carrier). Four immunization regimens were used. The first regimen included injection with 100 μg of DNPBGG in phosphate-buffered saline incorporated in Freund's complete adjuvant (FCA). The second regimen included injection with 100 μg of DNP-Lys-Ficoll incorporated in FCA. The third regimen included injection with PBS in FCA (sham-immunized) and the fourth regimen included

injection of PBS alone (non-immunized). Two to seven normal, sham-thymectomized or thymectomized rats (60 days of age) were subjected to each of the above immunization regimens.

Antibody analysis

Antibody activity to DNP in serum and saliva samples (n = 2–7) from each group obtained on days 4, 10, 16, 23, 30 and 38 after immunization was measured by an indirect enzyme linked immunosorbent assay (ELISA; Ebersole, Taubman & Smith, 1978b). Blood was collected from the retro-orbital sinus and serum obtained after clotting and centrifugation. Pilocarpine-stimulated (1 mg/100 g body weight) whole saliva was collected over a 20 min interval from each of the rats. For the ELISA technique, DNP was conjugated to calf thymus histone (HIS; Sigma) (Eisen *et al.*, 1953) and the molar ratio determined spectrophotometrically as described for DNP. The concentration of histone was determined by fluorescamine assay (Udenfriend, Stein, Boklen, Daiman, Leimgruber & Weigele, 1973). The final conjugate was found to contain 1·3 moles of DNP/mole of HIS. Three micrograms of DNP-HIS were attached to the wells of the microtitre plates and the system was developed with monospecific rabbit anti-rat μ chain globulin (Ebersole *et al.*, 1979) followed by reaction with goat anti-rabbit gamma globulins conjugated to alkaline phosphatase (Sigma). The substrate (*p*-nitrophenylphosphate) was added and the extent of reaction determined at 400 nm. Preliminary studies determined that the linear range of adsorption in this system required at least a 1 : 10 dilution of saliva and a 1:50 dilution of serum, both of which were subsequently utilized in the analyses. Serum IgM antibody levels are expressed as a ratio of absorbances at 400 nm between the experimental serum and a serum standard prepared by four injections of $DNP_{9\cdot6}$ BGG (Eisen *et al.*, 1953) emulsified in FCA into the salivary gland vicinity of three rats. Seven days following the last injection, the rats were exsanguinated by cardiac puncture and the serum obtained from clotted blood. Aliquots were stored at $-20°$. Similar to the experimental samples, dilutions of the serum standard giving a linear absorbance were included in each plate in triplicate. The mean reaction of the standard was determined and used in the data analysis. Salivary IgM antibody concentrations are expressed as A_{400nm} due to the unavailability of an appropriate saliva standard.

RESULTS

As we have previously reported, neonatal thymectomy significantly diminishes salivary IgA levels in thymectomized (Tx) rats relative to non-thymectomized rats, while IgG levels are generally unaffected (Ebersole *et al.*, 1979). IgM is detected in more than 50% (8/15) of the rats that had undergone neonatal thymectomy (Table 1). Thirty-six saliva samples from the fifteen rats (obtained from 28 to 75 days of age) were analysed for IgM. Of the thirty-six samples, fourteen contained detectable levels of IgM. In contrast, IgM was never detected in the salivas (sixty-eight samples) from rats in the two control groups (normal, N; sham-thymectomized, STx) throughout the 54 day sampling period. Serum IgM levels were similar among the N, STx and Tx rats (Table 1). Thus, neonatal thymectomy resulted in decreased levels of IgA (Ebersole *et al.*, 1979) coupled with the appearance of IgM in saliva. These data seem to suggest that a selective exocrine IgM compensation occurs in the saliva following neonatal thymectomy. In order to explore the effect of immunization on this IgM compensation, as well as the characteristics of the IgM antibody response in saliva, neonatally thymectomized and control rats were immunized with either a thymus-dependent (DNPBGG) or with a thymus-independent (DNP-Lys-Ficoll) antigen in the vicinity of the major salivary glands (Taubman & Smith, 1974). Theoretically, both types of antigens could induce a compensatory IgM antibody response in the thymectomized rats since this Ig class is least affected by the absence of thymus tissue (Cooper, Kincade, Bockman & Lawton, 1973). As suggested only the Tx rats demonstrate a salivary IgM antibody response after local injection of the antigens. The levels of salivary IgM antibody in the thymectomized rats appeared to be a function of whether a thymus-dependent ($A_{400nm} = 0\cdot36 \pm 0\cdot02$) or thymus-independent ($A_{400nm} = 0\cdot25 \pm 0\cdot05$) antigen was administered (Table 2). Salivary IgA anti-DNP activity was never detected during the sampling period in the Tx rats immunized with DNPBGG (Table 2), while all DNP-Lys-Ficoll injected Tx rats exhibited IgA antibody in their salivas. The local IgM response to DNPBGG (thymus-dependent) in the Tx rats was first observed on day 16. More than 70% (17/24) of the salivas, obtained from days 16 through 38, were positive for IgM anti-DNP antibodies. By day 23 all of the DNPBGG immunized Tx rats (6/6) showed salivary IgM antibodies to DNP. Local immunization with DNP-Lys-Ficoll (T-independent) also elicited salivary IgM antibody in Tx rats beginning on day 16 post-immunization. By day 38, 46·4% (13/28) of the samples were positive for IgM antibody (Table 2). Also,

the mean peak levels of salivary IgA antibody in Tx animals were reduced by at least 50% when compared with salivary IgA antibody levels in control (N, STx) animals immunized with DNP-Lys-Ficoll (Table 2). These findings appear to fulfill criteria for a compensatory antibody response, where the lack, or partial reduction, of IgA in secretions is compensated for by the local production and secretion of IgM antibodies.

Immunization of control animals with either antigen resulted in detectable IgM antibodies in only a few saliva samples (3/24) from normal rats (Table 2). IgM salivary antibodies were never detected in salivas from immunized sham-thymectomized (STx) rats during the sampling period (0/12). Only two of twenty saliva samples from thymectomized rats injected with adjuvant alone contained detectable IgM anti-DNP antibodies (Table 2) possibly as a result of previous exposure to certain cross-reactive antigens (Eisen, Michaelides, Underdown, Schulenburg & Simms, 1970). In the serum, similar concentrations of IgM

Table 1. Frequency of detection of IgM in saliva samples from normal, sham-thymectomized and thymectomized rats

Group	No. of rats pos./total	No. of salivas pos./ total samples tested*	Mean saliva IgM level of positive samples (μg/ml) ± SE	Mean serum IgM level (μg/ml) ± SE†
N	0/19	0/50	—	849 ± 45
STx	0/13	0/18	—	823 ± 33
Tx	8/15	14/36	7·6 ± 1·4	827 ± 48

* Saliva samples analysed were obtained from twenty normal (N), fifteen sham-thymectomized (STx) and seventeen thymectomized (Tx) rats 28, 35, 50 and 75 days of age.

†Serum samples from ten N and STx and seven Tx rats at 75 days of age.

Table 2. Salivary IgM anti-DNP antibodies in N, STx and Tx rats

Immunizing antigen	Group	No. of rats pos./total*	No. of pos./total No. of saliva samples tested*	Salivary IgM antibody†	Salivary IgA antibody†
DNPBGG	N	2/6	3/24	0·16	++‡
	STx	0/3	0/12	—	++
	Tx	6/6	17 24	0·36	—
DNP-Lys-	N	2/6	3/24	0·17	++
Ficoll	STx	0/3	0/12	—	++
	Tx	5/7	13/28	0·25	+
Sham	N	0/5	0/20	—	—
immunized§	STx	0/2	0/8	—	—
	Tx	1/5	2/20	0·18	—
Non-	N	0/5	0/20	—	—
immunized¶	STx	0/3	0/12	—	—
	Tx	0/4	0/16	—	—

*Data obtained from rat saliva samples on days 16–38 following immunization (results obtained from salivas on days 4 and 10 were uniformly negative for IgM antibodies). Any values which were greater than two standard deviations ($A_{400nm} = 0{\cdot}06$) from the mean 0·2 of all non-immunized rats were considered positive.

†Means determined from highest reactivity (A_{400nm}) that salivas from each positively responding rat attained during days 16–38.

‡(++) denotes a mean of ≥47 μg/ml of antibody; (+) 6–21 μg/ml of antibody; and (−) <5 μg/ml of antibody in each of the respective groups (salivas obtained from days 4–38).

§Denotes sham-immunized injected with PBS and FCA.

¶Denotes non-immunized injected with PBS.

Table 3. Serum IgM antibody responses in N, STx, and Tx rats after local immunization with DNPBGG and DNP-Lys-Ficoll

Immunizing antigen	Group	Peak level of antibody* (Experimental serum A_{400nm} / Standard serum A_{400nm})
DNPBGG	N	0·66 ± 0·06†
	STx	0·71 ± 0·06
	Tx	0·67 ± 0·05
DNP-Lys-Ficoll	N	0·44 ± 0·09
	STx	0·40 ± 0·08
	Tx	0·52 ± 0·06

* Rats from both the sham-immunized (SI) and non-immunized (NI) groups showed a background serum IgM response to DNP that remained at $A_{400nm} = 0·19 \pm 0·02$ throughout the experiment.

†Group mean ± SE of serums obtained on day 16 after immunization.

antibodies, peaking on day 16, were noted in all groups (N, STx and Tx) following local immunization with DNP-Lys-Ficoll or DNPBGG (Table 3). Therefore, the IgM antibody compensation appears to be limited to the exocrine secretions.

DISCUSSION

Previous investigations have found low levels of IgM in saliva of normal humans (Brandtzaeg *et al.*, 1968) or rodents (Ebersole *et al.*,1979); several studies in humans, indicated that the levels of IgM in salivary secretions of IgA-deficient subjects are frequently increased (Brandtzaeg *et al.*, 1968; Brandtzaeg, 1971; Savilhati, 1973; Arnold, Cole, Prince & McGhee, 1977) and that IgM seems to be actively transported into saliva (Brandtzaeg *et al.*, 1968). Local immunization of IgA- deficient humans has also led to the production of nasopharyngeal antibody of the IgM class (Ogra *et al.*, 1974). These results suggested that there may be a common mechanism for IgA and IgM secretion and that IgM may function as a compensatory immunoglobulin. The neonatally thymectomized rat was utilized as the model for exocrine IgA deficiency in this study. Examination of saliva from the thymectomized rats showed that nearly 40% of the samples contained detectable IgM levels. The present report examines parameters of this immunological replacement system, and suggests that this system provides a model to study the functional importance of this compensatory phenomenon. As shown in Table 1, serum IgM levels were similar among the groups, while salivary IgM was only detected in the Tx rats. In other experiments, groups of rats were injected with either DNPBGG or DNP-Lys-Ficoll in the hind foot pads and the Tx or control groups exhibited high serum IgM antibody levels with no detectable salivary IgM antibodies (J.L.E., M.A.T. and D.J.S., Submitted for publication). Also, in the experiments reported here, those rats that did develop a salivary IgM antibody response showed no correlation between individual serum and saliva samples with respect to either IgM levels or IgM antibodies to DNP. These results further support the concept that salivary IgM is locally synthesized, and could not be simply attributable to serum transudation. A significant negative correlation ($r = -0·605$, $P < 0·05$) between IgA and IgM immunoglobulin levels in individual control and Tx rats was noted and may suggest a possible homeostatic regulatory mechanism for secretory antibody between IgA and IgM secretion at the local site. This regulation could be explained by a direct competition for a similar transport mechanism (Brandtzaeg, 1971) leading to secretion of one or both of the immunoglobulin classes, or the levels may be a direct reflection of the relative numbers of cells synthesizing IgM and IgA in the glandular tissue. Experimental investigation of these two hypotheses may provide information concerning the control of IgA and IgM production and secretion into the external secretions.

In general, local immunization of rats with a variety of antigens results in specific exocrine antibodies of the IgA and IgG classes (Taubman & Smith, 1974; 1977; Ebersole, Taubman, Smith & Crawford, 1978a). Local immunization with DNPBGG (thymus-dependent) or DNP-Lys-Ficoll (thymus-independent) antigens results in similar levels of salivary IgG anti-DNP antibody, which are not significantly altered by neonatal thymectomy. On the other hand, removal of the thymus at birth severely impairs the salivary IgA response to DNP-Lys-Ficoll and reduces the salivary IgA response to DNPBGG to undetectable levels. This reduction in both cases seems to be partially compensated for by the appearance of salivary antibodies of the IgM class. This compensation is most pronounced after immunization with the thymus-dependent (DNPBGG) antigen when IgA is totally absent. It seems reasonable to assume that most major environmental antigens to which a host is exposed via mucous surfaces have T-dependent qualities. Although secretory IgA would normally constitute the

predominant antibody to these antigens, this study suggests that under conditions of T-cell deprivation and diminished IgA synthesis, IgM would comprise a portion of the immune response in secretions to this type of antigen. Therefore, it is suggested that local immunization with T-dependent antigens of a microorganism, followed by an infectious challenge with the same agent could constitute a model to study functional aspects of the compensatory IgM reponse in secretions. In addition, certain environmental antigens (e.g., lipopolysaccharide, dextran, levan and flagellin) (Miller & Osoba, 1967) have T-independent characteristics. The results obtained in this study indicate that the T-independent antigens may also evoke both a reduced IgA response and a compensatory IgM response in situations of depleted T-cell numbers. Therefore, the neonatally thymectomized rat can serve as a model to study the effects of IgM compensation after immunization with thymus-dependent or thymus-independent antigens.

The importance of the protective capacity of IgA in external secretions of the respiratory and intestinal tract, and of the oral cavity has been supported by numerous investigations (Taubman & Smith, 1974, 1977; Michalek, McGhee, Mestecky, Arnold & Bozzo, 1976). In a pilot study, Cole, Arnold, Rhodes & McGhee (1977) have suggested that salivary IgM compensation in certain IgA-deficient humans may provide a measure of protection against dental caries. These results have recently been supported in a more extensive study of IgA-deficient individuals, where IgM compensation was noted in saliva of 32% of the patients (Arnold *et al.*, 1977). This compensation resulted in agglutinin levels to *Streptococcus mutans* that were similar to normal saliva samples. The present observations in the thymectomized rat confirm that, at mucosal surfaces, there is an IgM compensatory immune response accompanying IgA deficiency. The functional significance of such a response, however, has not been determined. The model proposed should enable determination of the ability of IgM antibody to provide protection from bacterial infections after active immunization or natural exposure.

ACKNOWLEDGMENTS

We thank Deirdre Frey and Julia Buckelew for expert technical assistance. Also, the suggestions and discussions of Drs Robert J. Genco and Ronald J. Gibbons pertaining to the manuscript are gratefully acknowledged.

This work was supported by a contract (DE-42438) and grant (DE-03420) from the National Institute of Dental Research. DJS (DE-00024) and JLE (DE-00075) are recipients of Public Health Service Research Career Development Awards.

REFERENCES

ARNOLD R.R., COLE M.F., PRINCE S. & MCGHEE J.R. (1977) Secretory IgM antibodies to *Streptococcus mutans* in subjects with selective IgA deficiency. *Clin. Immunol. Immunopathol.* **8,** 475.

BRANDTZAEG P. (1971) Human secretory immunoglobulins. II. Salivary secretions from individuals with selectively excessive or defective synthesis of serum immunoglobulins. *Clin. exp. Immunol.* **8,** 69.

BRANDTZAEG P., FJELLANGER I. & GJERULDSEN S.T. (1968) Immunoglobulin M: local synthesis and selective secretion in patients with immunoglobulin A deficiency. *Science,* **160,** 789.

BRANDTZAEG P. & TOLO K. (1977) Mucosal penetrability enhanced by serum derived antibodies. *Nature (Lond.),* **266,** 262.

COLE M.F., ARNOLD R.R., RHODES M.J. & MCGHEE J.R. (1977) Immune dysfunction and dental caries: a preliminary report. *J. dent. Res.* **56,** 198.

COOPER M.D., KINCADE P.W., BOCKHAM D.E. & LAWTON A.R. 1973) Origin, distribution and differentiation of IgA-producing cells. *Adv. exp. Biol. Med.* **45,** 13.

CRABBÉ P.A., CARBONERA A.O. & HEREMANS J.F. (1965) The normal human intestinal mucosa as a major source of plasma cells containing γ A- immunoglobulin. *Lab. Invest.* **14,** 235.

CYPESS R.H., EBERSOLE J.L. & MOLINARI J.A. (1977) Specific antibody levels in the intestinal perfusates of *Heligosomoides polygyrus* infected mice. *Int. Arch. Allergy Appl. Immunol.* **55,** 496.

DUBOIS M., GILLES K.A., HAMILTON J.K., ROBERS P.A. & SMITH F. (1956) Colorimetric method for determination of sugars and related substances. *Anal.. Chem.* **28,** 350.

EBERSOLE J.L., TAUBMAN M.A., SMITH D.J. & CRAWFORD J.M. (1978a) Characterization of immunoglobulin-containing cells in the submandibular gland of the rat after local immunization. *Adv. exp. Biol. Med.* **107,** 155.

EBERSOLE J.L., TAUBMAN M.A. & SMITH D.J. (1978b) Thymic control of secretory antibody responses. *J. dent. Res.* **57,** A104.

EBERSOLE J.L., TAUBMAN M.A. & SMITH D.J. (1979) The effect of neonatal thymectomy on the level of salivary and serum immunoglobulins in rats. *Immunology,* **36,** 649.

EISEN H.N., BELMAN S. & CARSTEN M.E. (1953) The reaction of 2,4-dinitrobenzenesulfonic acid with free amino groups of proteins. *J. Am. chem. Soc.* **75,** 4583.

EISEN H.N., MICHAELIDES M.C., UNDERDOWN B.J., SCHULENBURG E.P. & SIMMS, E.S. (1970) Myeloma proteins with anti-hapten antibody activity. *Fed. Proc.* **29,** 78.

MANCINI G., CARBONERA A.O. & HEREMANS J.F. (1965) Immunochemical quantitation of antigens by single radial immunodiffusion. *Immunochemistry,* **2,** 235.

McMaster P.R.B., Owens J.D. & Vannier W.E. (1977) The preparation and characterization of a thymic independent antigen: ε-dinitrophenyl-L-lysine-Ficoll. *Immunochemistry*, **14**, 189.

Michalek S.M., McGhee J.R., Mestecky J.M., Arnold R.R. & Bozzo L. (1876) Ingestion of *Streptococcus mutans* induces secretory immunoglobulin A and caries immunity. *Science*, **192**, 1238.

Miller J.F.A.P. (1960) Studies on mouse leukemia. The role of the thymus in leukemogenesis by cell free leukemia filtrates. *Br. J. Cancer*, **14**, 93.

Miller J.F.A.P. & Osoba D. (1967) Current concepts of immunological function of the thymus. *Physiol. Res.* **47**, 437.

Ogra P.L., Coppola P.R., MacGillivray M.H. & Dzierba J.L. (1974) Mechanism of mucosal immunity to viral infections in γ A immunoglobulin-deficiency syndromes. *Proc. Soc. exp. Biol. Med.* **145**, 811.

Rockey J.H., Hanson L.A., Heremans J.F. & Kunkel H.G. (1964) Beta-2A-aglobulinemia in 2 healthy men. *J. lab. clin. Med.* **63**, 205.

Savilhati E. (1973) IgA deficiency in children. Immunoglobulin-containing cells in the intestinal mucosa, immunoglobulins in secretions and serum IgA levels. *Clin. exp. Immunol.* **13**, 395.

Sharon R., McMaster P.R.B., Kask A.M., Owens J.D. & Paul W.E. (1975) A T-independent antigen which elicits both IgM and IgG anti-DNP antibody-secreting cells. *J. Immunol.* **114**, 1585.

Taubman M.A. & Smith D.J. (1974) Effects of local immunization with *Streptococcus mutans* on induction of salivary IgA antibody and experimental dental caries in rats. *Infect. Immun.* **9**, 1079.

Taubman M.A. & Smith D.J. (1977) Effects of local immunization with glucosyltransferase fractions from *Streptococcus mutans* on dental caries in rats and hamsters. *J. Immunol.* **118**, 710.

Tomasi T.B. & Bienenstock J. (1968) Secretory immunoglobulins. *Adv. Immunol.* **9**, 1.

Tourville D.R., Adler R.H., Bienenstock J. & Tomasi T.B. (1969) The human secretory immunoglobulin system: immunohistological localization of γ A, secretory 'piece', and lactoferrin in normal human tissues. *J. exp. Med.* **129**, 411.

Udenfriend S., Stein, S., Boklen P., Daiman, W., Leimgruber W. & Weigele M. (1973) Fluorescamine: a reagent for assay of amino acids, peptides, proteins and primary amines in the picomole range. *Science*, **178**, 871.

Waldman R.H. & Ganguly R. (1974) Immunity to infections on secretory surfaces. *J. infect. Dis.* **130**, 419.