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Marmoset species variation in the humoral antibody response: *in vivo* and *in vitro* studies

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Summary. A comparison of the *in vivo* and *in vitro* antibody response capabilities of two marmoset species, *Saguinus fuscicollis* and *Saguinus oedipus oedipus*, revealed the former to be superior in elaborating humoral antibody. *In vivo* challenges with *Escherichia coli* lipopolysaccharide (LPS) and *Salmonella typhi* flagella consistently yielded higher antibody titres in *S. fuscicollis*; indeed, with LPS antigen, multiple inoculations of *S.o. oedipus* marmosets led ultimately to a decrease in antibody formation, in contrast to the anamnestic response of *S. fuscicollis*. This species differential in immune competence was also suggested in the *in vitro* stimulation of peripheral blood leucocytes (PBL) and spleen cells with sheep red blood cells (RBC). None of 55 *S.o. oedipus* PBL cultures and 49 of 89 (55%) *S. fuscicollis* cultures responded to the test antigen. A similar differential in response to sheep RBC was noted with the spleen cells of each species. Although this report contrasts the antibody-forming potential of two marmoset species, a comparison of the immunological response profile of marmosets to those of other laboratory animals challenged with similar antigens suggests these primates may be relatively incompetent. The possible relationship between the haemopoietic chimerism of marmosets and a diminished immune competence is discussed.

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INTRODUCTION

Marmosets, small New World primates belonging to the Callithricidae family, are unique in that they consistently produce dizygotic twins (Hill, 1932; Wislocki, 1939). Further, placental vascular anastomoses between the developing embryos lead to haemopoietic chimerism and immunological tolerance among the co-twins (Wislocki, 1939; Benirschke, Anderson & Brownhill, 1962; Gengozian, Batson & Eide, 1964; Gengozian, Batson, Greene & Gosslee, 1969; Porter & Gengozian, 1969; Porter & Gengozian, 1973). We have conducted basic studies on the immunological potential of these natural-born chimeras in an attempt to gain an understanding of the tolerance-inducing mechanism. Two marmoset species, *Saguinus fuscicollis* and *Saguinus oedipus oedipus*, bred and maintained in our laboratory, have been studied for both cell-mediated and humoral response capabilities. Surprisingly, a

species distinction in their ability to respond to sheep red blood cells (RBC) was noted, while skin allotransplantation and mixed lymphocyte culture (MLC) reactions have failed to reveal a difference (Porter & Gengozian, 1973; Gengozian, Salter, Basford & Kateley, 1976; Barnhart & Gengozian, 1975; Gengozian & Porter, 1971). In the humoral immune response to sheep RBC, *S.o. oedipus* marmosets consistently yielded lower agglutinin and haemolysin titres, independent of the route of immunization and number of antigenic challenges (Gengozian, Salter, Basford & Kateley 1976). An interesting characteristic feature of the response in each species, however, was the failure to produce 7S haemolysins following multiple intravenous inoculations although immunological memory was evident in animals given the antigen intramuscularly. That the immunological potential of marmosets be analysed further is dictated by other reports suggesting that the immune system of these animals may be compromised. Thus, Deinhardt (1972) has reported both species to be readily susceptible to oncogenic viruses, while Harvey, Felsburg, Heberling, Kniker & Kalter (1974) noted that *S.o. oedipus* produced a meagre antibody response to typhoid and influenza vaccines when compared to three other subhuman (non-marmoset) primates.

Since our previous studies had not revealed any species differential in response to several immune functions elaborated by T cells, i.e. skin allograft rejections, MLC reactions and mitogen stimulation, we considered the thesis that the subnormal response of *S.o. oedipus* marmosets to sheep RBC may be due to a deficiency in the B-cell compartment. To explore this possibility, we tested the ability of both species to respond to *Escherichia coli* lipopolysaccharide, a T-independent antigen (Andersson & Blomgren, 1971; Möller & Michael, 1971). Additional studies were also conducted with the T-dependent antigens, *Salmonella typhi* flagella and sheep RBC, to provide further information on the immune response profile of marmosets. The results of both *in vivo* and *in vitro* assays again reveal *S.o. oedipus* marmosets to be subnormal for humoral antibody formation when compared to *S. fuscicollis*.

MATERIALS AND METHODS

Animals

Two species of marmosets were studied, *S.o. oedipus* and *S. fuscicollis*. Imported and laboratory-bred animals were used randomly, with no significant effects in any experiment being attributable to the animal source. The ages of the imported animals were not known; however, all had been in our colony at least 12 months and therefore, based on our breeding studies, were considered to be sexually mature (Gengozian, Batson & Smith, 1978). Laboratory-bred marmosets ranged in ages from 1·5 years to 4·5 years and weights of all animals ranged from 350 to 450 g. Maintenance and handling procedures have been described elsewhere (Gengozian, 1969).

In vivo studies

Two antigens were used: (a) *Escherichia coli* lipopolysaccharide (LPS, 0127B : 8; Difco, Detroit, Michigan) in concentrations of 5 μg or 50 μg per animal, and *Salmonella typhi* flagella at 5 μg. The antigens were administered either intravenously (i.v.) in the femoral vein or intramuscularly (i.m.) in the outer aspects of the thigh. All animals received two booster inoculations (same antigen dose) at 50-day intervals via the route of the initial challenge. Animals were bled at regular intervals and the sera stored at $-30°$ until tested.

To detect bacterial agglutinins, two fold serial dilutions of individual serum samples were made in 0·1 ml of phosphate buffered saline (PBS, pH 7·2). The passive haemagglutination technique with LPS-sensitized marmoset erythrocytes was used to assess anti-LPS antibody (Friedman, Allen & Rosenzweig, 1969). An equal volume of target erythrocytes and diluted serum was incubated at 37° for 1 h after which the tubes were centrifuged and the agglutination determined macroscopically. Anti-typhoid agglutinins were assayed by the typhoid H antigen (flagella) agglutination reaction. An equal volume of the particulate antigens (Markham Laboratories, Chicago, Illinois) was added to the diluted serum; the mixture was incubated at 56° for 2 h and at 4° overnight. Agglutination was scored by the sedimentation pattern. Titres for both anti-LPS and anti-flagella antibodies were expressed as $\log_2$ of the reciprocal of the highest dilution showing agglutination.

To determine the molecular species of antibody formed, antiserum was combined with 2-mercaptoethanol (2-ME) and PBS to give a final 2-ME concentration of 0·1 M. Incubation of this mixture at 37° for 1 h was followed by serial dilution in PBS. Antigen was added to each tube and incubated

as in the total antibody tests. This procedure permitted determination of 2-ME sensitive and 2-ME resistant antibody, hereafter referred to as 19S and 7S antibody, respectively. Sucrose density gradient and G-200 Sephadex column chromatography verified the size distinctions made for these two classes of antibody (Gengozian *et al.*, 1976).

***In vitro* studies**

Peripheral blood leucocytes (PBL) were isolated from defibrinated blood on an Isopaque-Methocel gradient (Barnhart & Gengozian, 1975) and cultures for *in vitro* antibody formation were prepared as described by Nickerson & Gengozian (1976). Briefly, 1×10^6 PBL in 1 ml of RPMI-1640, supplemented with 10% foetal calf serum (FCS; Reheis, Stock No. 268, Control No. N41801), 100 U/ml penicillin and 100 mcg/ml streptomycin were placed in glass culture tubes (16×100 mm). Sheep RBC (1×10^5, 1×10^6, or 1×10^7) were added to experimental cultures in 0·05 ml of supplemented RPMI-1640. Cultures were maintained at 37° in a humidified atmosphere of 5% CO_2 and air. Cells were harvested for determination of plaque-forming cells (PFC) on day 9, this time period having been found to be optimal for maximum PFC development (Nickerson & Gengozian, 1976).

Spleens were obtained aseptically. Cell suspensions were prepared by gently squeezing the fragmented spleen through a fine nylon mesh in Hanks's balanced salt solution (HBSS). This suspension was washed once in HBSS. Spleen cells were cultured by two methods: (a), by the tube culture technique described above for PBL, using only 1×10^6 nucleated spleen cells per tube, and (b), by the Mishell-Dutton technique (Mishell & Dutton, 1967) as modified by Cosenza, Leserman & Rowley (1971). In the latter procedure, 10×10^6 spleen cells in 1 ml of minimum essential medium were placed in plastic petri dishes (35×10 mm) and sheep RBC (1×10^6, 1×10^7, or 1×10^8) added to appropriate experimental cultures. Petri dishes were maintained in

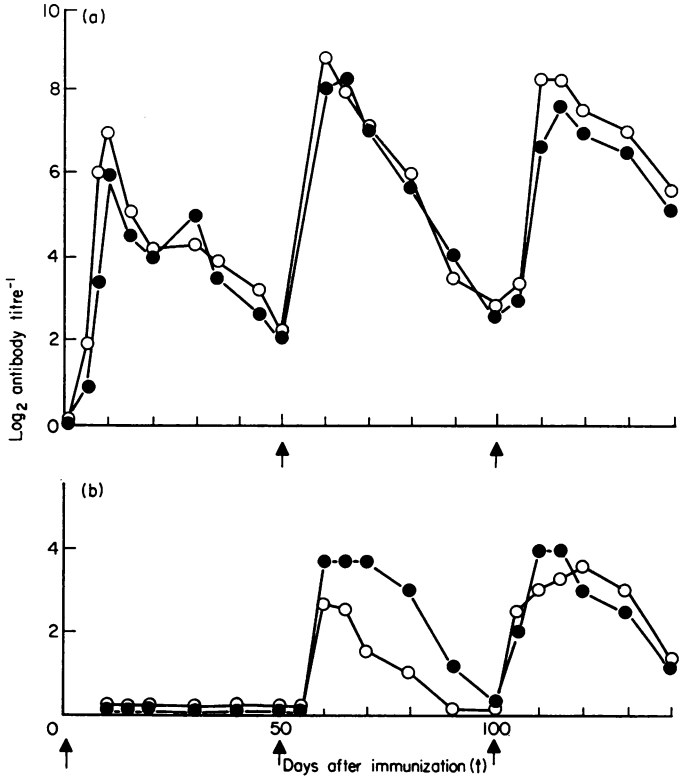

Figure 1. Response of *S. fuscicollis* marmosets to 5 μg or 50 μg of *E. coli* LPS given intravenously. (○) 50 μg; (●) 5 μg. (a) Total antibody, (b) 2-ME resistant antibody.

lucite boxes in an atmosphere of 7% O_2, 10% CO_2, and 83% N_2 at 37°. The boxes were rocked continuously on a rocker platform and fed daily with 0·05 ml FCS and 0·1 ml nutritional mixture (Cosenza *et al.*, 1971). Cultures from both methods were harvested after 5 and 7 days and PFC determined.

The cultured cells were assayed for direct (19S) PFC by the slide modification of the Jerne technique (Jerne & Nordin, 1963; Plotz, Talal & Asofsky, 1968). Conditions for optimal development of plaques with marmoset antibody-producing cells and sheep RBC antigen have been previously reported (Gengozian *et al.*, 1976).

RESULTS

In vivo studies

Eight *S. fuscicollis* marmosets were inoculated i.v. with *E. coli* LPS, four receiving 5 μg and four receiving 50 μg (Fig. 1). No antigen dose effect within this range was observed and the mean anti-LPS serum titres in the two groups were almost identical throughout the primary, secondary and tertiary responses with respect to both 19S and 7S agglutinins. Although there was variation in the responses of individual marmosets, the mean antibody titres between groups never differed by more than one $\log_2$ unit.

Four *S.o. oedipus* marmosets were inoculated i.v. with 50 μg *E. coli* LPS and their serum agglutinin titres compared to that of *S. fuscicollis* receiving a similar dose (Fig. 2). The agglutinins detected during the primary response in both species were all 2-ME sensitive, i.e. 19S antibody. The titres in *S.o. oedipus*, however, were considerably lower than that obtained for *S. fuscicollis* marmosets during the 50-day observation period, with the former group showing a mean peak titre of 4 ($\log_2^{-1}$) compared to 7 for *S. fuscicollis*.

Additional contrasts in the response of these two species were evident after a second and third LPS challenge. *S. fuscicollis* responded anamnestically after the second injection, while the titres in *S.o. oedipus* did not exceed that observed during the

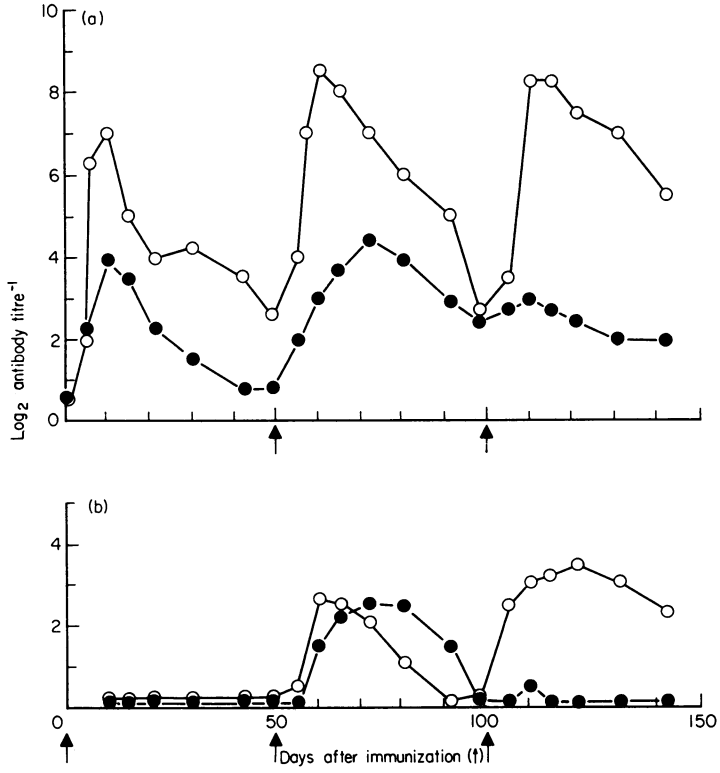

Figure 2. A comparison of the response of *S. fuscicollis* and *S.o. oedipus* marmosets to 50 μg of *E. coli* LPS given intravenously. (○) *S. fuscicollis*; (●) *S.o. oedipus*. (a) Total antibody, (b) 2-ME resistant antibody.

primary response. More significantly, perhaps, the third antigen inoculation of *S.o. oedipus* gave a peak titre lower than that of either the primary or secondary, while in *S. fuscicollis* the response was comparable to that observed after the second injection. One of the four *S.o. oedipus* marmosets had detectable levels of 7S agglutinins in its serum during this period while all *S. fuscicollis* animals gave a small but significant response for this class of antibody. Although not shown in Fig. 2, a fourth inoculation of the *S.o. oedipus* animals yielded a further reduction in serum titres, never exceeding a mean value of 1·5 ($\log_2^{-1}$).

Two groups of four *S. fuscicollis* marmosets each were immunized with 5 μg *S. typhi* flagella, one receiving the antigen i.v. and the other i.m. The mean agglutinin titres in each immunization group were similar with respect to class and titre of agglutinins formed and the pattern of the antibody response (Fig. 3). A group of four *S.o. oedipus* marmosets were inoculated with 5 μg *S. typhi* flagella i.v. and their serum titres compared to that of the *S. fuscicollis* inoculated in the same manner (Fig. 4). Serum titres increased rapidly after each flagellar immunization and maximal titres in each species generally occurred 10–14 days following challenge. Again, *S. fuscicollis* marmosets responded with higher titres, particularly after the primary and secondary inoculations. It is interesting to note, however, that in these animals there was no obvious anamnestic response since the mean peak titres were approximately the same after each antigen challenge. Similarly, although 2-ME resistant antibodies appeared in the serum after each immunization, the titres did not increase significantly following the secondary or tertiary immunization. Although the *S.o. oedipus* animals showed a lower immune response profile throughout, their serum titres increased slightly following each immunization with the flagella antigen. 2-ME resistant antibodies

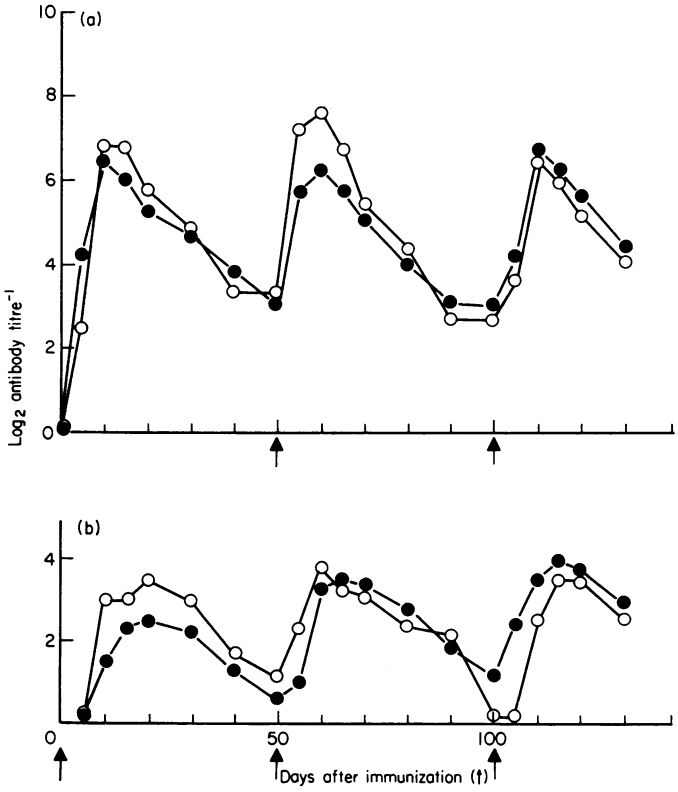

Figure 3. Response of *S. fuscicollis* marmosets to 5 μg *S. typhi* flagella antigen given intramuscularly or intravenously. (○) Intramuscular, (●) intravenous. (a) Total antibody, (b) 2-ME resistant antibody.

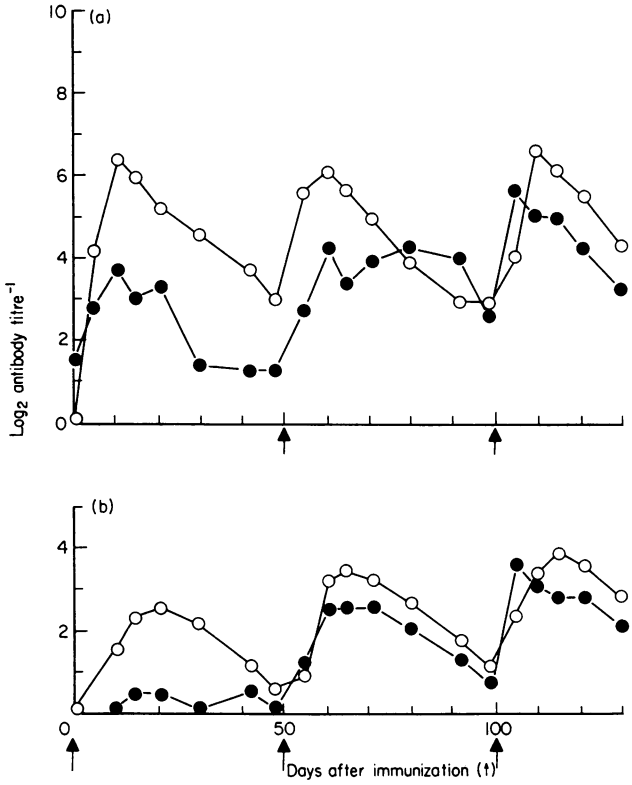

Figure 4. A comparison of the response of *S. fuscicollis and S.o. oedipus* marmosets to 5 μg of *S. typhi* flagella given intravenously. (○) *S. fuscicollis*; (●) *S.o. oedipus*. (a) Total antibody, (b) 2-ME resistant antibody.

in this species showed a similar pattern of appearance, barely detectable in the primary but increasing after the second and third inoculation.

In vitro studies

We have previously reported successful antibody formation with blood leucocytes of marmosets when these were cultured *in vitro* with sheep RBC antigen (Nickerson & Gengozian, 1976). The objective of that study was to demonstrate that a readily available tissue could be used for immunological studies in primates or humans, species from which the more commonly used tissues such as lymph nodes and spleen are difficult to obtain. In testing a limited number of *S. fuscicollis* and *S.o. oedipus* marmosets, however, it was found that blood samples from the latter species failed to respond while 80% of those from *S. fuscicollis* yielded significant numbers of PFC to sheep RBC. To determine whether this species differential was again real with respect to this mode of response, blood leucocytes from 89 *S. fuscicollis* marmosets and 55 *S.o. oedipus* were cultured with sheep RBC and the PFC determined. Each leucocyte sample was cultured with three different concentrations of sheep RBC (see Materials and Methods) and the highest number of PFC obtained with either of the three ratios of antigen to PBL has been utilized for the data given in Table 1. None of the 55 *S.o. oedipus* cultures showed any PFC, while 49 of the 89 (55%) *S. fuscicollis* cultures yielded a positive response. As shown in Table 1, the number of PFC per 1×10^6 cultured leucocytes ranged from 50 to greater than 1500; while the majority of cultures yielded less than 500 $PFC_p/1 \times 10^6$ cells, 23 of the 49 responding cultures gave values greater than this.

To verify that this species differential in *in vitro* antibody formation was not due to some unknown variability in the spectrum of cell types to be found in the peripheral blood, we cultured splenic cells of each marmoset species with sheep RBC and de-

Table 1. Primary *in vitro* response to sheep RBC by blood leucocytes of *S. fuscicollis* and *S.o. oedipus* marmosets

Species	No. cultures responding / No. cultures tested	PFC/10^6 cells* Range of response	Number of animals
S. fuscicollis	49/89	50–250	18
		251–500	8
		501–750	9
		751–1000	5
		1001–1500	4
		1501–2000	5
S.o. oedipus	0/55	—	—

* Values represent 19S plaque-forming cells (PFC) per 10^6 harvested leucocytes. Background PFC activity, i.e. from cultures not containing sheep RBC, have ranged from 0 to 22 PFC/10^6 harvested leucocytes.

termined the number of PFC formed. As noted in the Materials and Methods, two techniques were used, one utilizing tube cultures of only 1×10^6 nucleated cells, similar to the procedure with the blood leucocytes described above, and the other the classical Mishell-Dutton technique with 10×10^6 cells in plastic petri dishes. The results, shown in Table 2, again indicate a greater potential of *S. fuscicollis* tissue to respond to this antigen. The single *S.o. oedipus* spleen yielding PFC did so in only a marginal fashion, while all four *S. fuscicollis* spleens gave greater than 100 PFC/10^6 cultured cells. It is interesting to note that in each case the numbers of PFC formed were consistently greater with the tube-type technique in which cultures were initiated with only 1×10^6 cells. Finally, as observed in our previous and present studies with PBL, no optimal ratio of antigen to spleen cells for maximum PFC development was apparent (Nickerson & Gengozian, 1976).

Table 2. Primary *in vitro* response to sheep RBC by spleen cells of *S. fuscicollis* and *S.o. oedipus* marmosets

Species and animal no.	Mishell-Dutton culture*						Tube-culture*					
	Day 5†			Day 7			Day 5			Day 7		
	1 : 10‡	1 : 1	10 : 1	1 : 10	1 : 1	10 : 1	1 : 10	1 : 1	10 : 1	1 : 10	1 : 1	10 : 1
S. fuscicollis												
1.	27§	19	48	5	5	54	288	127	350	1269	561	1212
2.	8	12	8	64	60	84	192	339	233	109	764	1556
3.	0	0	0	0	0	0	—	—	—¶	121	345	145
4.	0	0	0	0	0	0	26	191	0	0	0	0
S.o. oedipus												
1.	0	0	0	0	0	0	0	0	0	0	0	0
2.	0	0	0	0	0	0	0	0	0	0	0	0
3.	0	0	0	0	0	0	0	0	0	0	0	0
4.	15	22	6	6	19	0	93	16	47	51	29	0

* As noted in the Materials and Methods, the Mishell-Dutton culture was initiated with 10×10^6 cells in plastic petri dishes and the tube culture was initiated with 1×10^6 cells.

† Indicates day of culture on which cells were harvested for PFC determination.

‡ Indicates ratio of sheep RBC to spleen cells in culture.

§ Values represent 19S plaque-forming cells (PFC) per 10^6 harvested cells.

¶ Not done.

DISCUSSION

The present data, in agreement with our earlier studies using sheep RBC (Gengozian *et al.*, 1976), emphasize inherent differences in the humoral response capabilities of the two marmoset species, *S. fuscicollis* and *S.o. oedipus*. The former animals showed a consistently higher response to injections of either *S. typhi* flagella or *E. coli* LPS, and *in vitro* stimulation of PBL or spleen cells with sheep RBC also revealed a greater immune potential of *S. fuscicollis*. In addition to a species variation in the titres of antibody formed, the immune response profile of *S.o. oedipus* differed markedly from that of *S. fuscicollis*. For example, continued stimulation of *S.o. oedipus* with LPS led ultimately to a decrease in antibody titres, with a fourth injection of this antigen yielding only a marginal response. Whether this represents a species susceptibility on the part of *S.o. oedipus* toward immunological paralysis with this antigen is not known but the observations are in contrast to the results obtained with *S. fuscicollis*. *E. coli* LPS was chosen for the present study because of its reported independence of T cells for the elaboration of humoral antibody (Andersson & Blomgren, 1971; Möller & Michael, 1971) and our previous findings indicating that a variety of T-cell functions are not noticeably different in the two species. Thus, skin allotransplants, MLC reactions, and stimulation of PBL by T-cell mitogens have revealed *S.o. oedipus* to be as responsive as *S. fuscicollis* (Porter & Gengozian, 1973; Barnhart & Gengozian, 1975; Gengozian & Porter, 1971; Kateley, Nickerson & Gengozian, 1977). The overall lower response of the *S.o. oedipus* marmosets to LPS could suggest an impairment in the B-cell population, e.g. a defect in the ability of these cells to synthesize and/or release immune globulins. Recent studies, however, have shown that LPS may influence the physiological activities of T cells which in turn may modulate B cell function. Thus, in examining the lymphoid cells which may serve as targets for LPS stimulation, Shinohara & Kern (1976) reported that whereas LPS induced proliferation of B cells, T cells regulated differentiation of B cells into immunoglobulin-producing cells. Although the latter study was concerned with antigen-independent immunoglobulin production by LPS stimulation, the absence of any T-cell influence on antibody formation to LPS in the marmoset cannot be assumed, particularly in view of the fact that T cells were not eliminated from our system. Further studies with known T-independent antigens in experimental designs wherein the T cell input can be controlled are needed before we can explain the difference in humoral response capabilities of the two marmoset species on the basis of T-B cell competence or collaboration.

The *in vitro* tests with sheep RBC, using either PBL or spleen cells as targets for stimulation, parallel our earlier *in vivo* studies with this antigen showing a lesser response by *S.o. oedipus* marmosets. Indeed, with PBL, a response by *S.o. oedipus* cells has been nonexistent. Human PBL have also been reported to respond to sheep RBC *in vitro* although the degree of success has varied in different laboratories. Thus, a non-predictable or variable PFC development similar to what we have observed with marmoset *S. fuscicollis* PBL has also been noted by Delfraissy, Galanaud, Dormont & Wallon (1977), while Dosch & Gelfand (1977), using different culture conditions and cell numbers, have found human PBL to be reliably responsive. The latter investigators also showed a relationship between antigen concentration and the numbers of T cells present in a given cell suspension required for maximum induction of PFC. That this was not a major factor in our study with marmoset PBL was the finding that even with antigen : PBL ratios ranging from 1 : 10 and 10 : 1, no one concentration of antigen was optimal. Allowance for this variable and the fact that peripheral blood T and B cell proportions are the same for the two marmoset species under investigation (Niblack & Gengozian, 1976) underscore further the negative results with *S.o. oedipus* PBL. Luzzati, Taussig, Meo & Pernis (1976), in their study with human PBL and sheep RBC, have suggested the presence of an inhibitory adherent cell, which when removed, permitted induction of a small antibody response. To determine whether such a cell population could account for the pronounced species differences noted here, peripheral blood from ten *S.o. oedipus* and ten *S. fuscicollis* marmosets were filtered on nylon wool as described by Luzzati *et al.* (1976) and the cells cultured with sheep RBC. The results were comparable to what has been consistently obtained with unfiltered blood, i.e. no response by any of the *S.o. oedipus* cultures and a variability in response of *S. fuscicollis* cells with no apparent enhancement in numbers of PFC. It should be noted that even with nylon wool filtration, positive results with human

PBL were obtained in only 50% of the cultures attempted (Luzzati *et al.*, 1976).

Although this report has emphasized a marmoset species differential in humoral antibody formation, a comparison of the marmoset's immune competence relative to other laboratory animals immunized with similar antigens may also be made. Thus, the induced agglutinin titres to LPS and flagellar antigens in this species, even that of *S. fuscicollis*, were considerably lower than those reported by other investigators using mice, rats, rabbits, and even humans (Jackson & Walters, 1972; Britton, 1969; Nossal, Ada & Austin, 1964; Humphrey, Parrott & East, 1964; Rowley & MacKay, 1969; Davies, Carter, Leuchars, Wallis & Dietrich, 1970). For example, repeated immunizations of *S. fuscicollis* marmosets with *S. typhi* flagella heightened neither the IgM nor IgG titres and did not evoke a true anamnestic response. Similar observations had been made previously in evaluating the agglutinin response of this species to sheep RBC antigens relative to that of other animals (Gengozian *et al.*, 1976). While such species comparisons are admittedly difficult to make and cannot form a basis for classifying animals as 'immunlogically compromised', it must be recognized that the antigens used in the studies cited are highly immunogenic in other laboratory species. Aside from any quantitative considerations or possible physiological defects in T and/or B lymphocytes in the marmoset, an important aspect concerning all immunological studies in this species is the chimeric nature of its haemopoietic elements. Studies in other chimeric animals, all laboratory derived, have revealed immunological abnormalities. Urso & Gengozian (1973; 1974) reported that murine allogeneic radiation chimeras which survive secondary disease often respond poorly to sheep RBC although these animals contain a normal percentage of T and B cells in the peripheral lymphoid organs. Even in the tetraparental mouse, the laboratory model which comes closest to the marmoset in chimerism induction and degree of chimerism, there are data which suggest a subtle but definite alteration in the responses engendered by the chimeric populations in this animal. Both the antigens and genetic strain combinations used to create the tetraparental animal are critical; thus, normal (Bechtol, Wegmann, Freed, Grumet, Chesebro, Herzenberg & McDevitt, 1974) and subnormal (Bechtol & McDevitt, 1976; Warner, Fitzmaurice, Maurer, Merryman & Schmerr, 1973) responses have been reported. The latter observations and our present findings have therefore prompted current *in vitro* studies to determine the influence of lymphocyte chimerism on the immune status of the marmoset and to identify the mechanism which could account for the differences in immune responsiveness between the two marmoset species (Gengozian, Bazzell & Nickerson, 1976).

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REFERENCES

ANDERSSON D.D. & BLOMGREN H. (1971) Evidence for thymus-independent humoral antibody production in mice against polyvinylpyrrolidone and *E. coli* lipopolysaccharide. *Cell. Immunol.* **2,** 411.

BARNHART D.D. & GENGOZIAN N. (1975) An evaluation of the mixed lymphocyte culture (MLC) reaction in marmosets. *Transplantation*, **20,** 107.

BECHTOL K.B. & MCDEVITT H.O. (1976) Antibody response of C3H↔(CKB × CWB)F_1 tetraparental mice to poly-$_L$(Tyr, Glu)-poly-D, $_L$-Ala--poly-$_L$-Lys immunization. *J. exp. Med.* **144,** 123.

BECHTOL K.B., WEGMANN T.G., FREED J.H., GRUMET F.C., CHESEBRO B.W., HERZENBERG L.A. & MCDEVITT H.O. (1974) Genetic control of the immune response to (T,G)-A--L in C3H↔C57 tetraparental mice. *Cell. Immunol.* **13,** 264.

BENIRSCHKE K., ANDERSON J.M. & BROWNHILL L.E. (1962) Marrow chimerism in the marmosets. *Science*, **138,** 513.

BRITTON S. (1969) Regulation of antibody synthesis against *Escherichia coli* endotoxin. II. Specificity, dose requirements and duration of paralysis induced in adult mice. *Immunology*, **16,** 513.

COSENZA H., LESERMAN L.D. & ROWLEY D.A. (1971) The third cell type required for the immune response of spleen cells *in vitro*. *J. Immunol.* **107,** 414.

DAVIES A.J.S., CARTER R.L., LEUCHARS E., WALLIS V. & DIETRICH F.M. (1970) The morphology of immune reactions in normal, thymectomized and reconstituted mice. III. Response to bacterial antigens: Salmonella flagellar antigen and pneumococcal polysaccharide. *Immunology*, **19,** 945.

DEINHARDT F. (1972) Induction of neoplasms by viruses in marmoset monkeys. *J. med. Primatol.* **1,** 29.

DELFRAISSY J.F., GALANAUD P., DORMONT J. & WALLON C. (1977) Primary *in vitro* antibody response from human peripheral blood lymphocytes. *J. Immunol.* **118,** 630.

DOSCH H.M. & GELFAND E.W. (1977) Generation of human plaque-forming cells in culture: tissue distribution, antigenic and cellular requirements. *J. Immunol.* **118,** 302.

FRIEDMAN H., ALLEN J. & ROSENZWEIG J. (1969) Comparison of bacteriolysis, passive hemolysis, and bacterial adherence colony formation for detecting antibody-forming cells. *Proc. Soc. exp. Biol. Med.* **131,** 353.

GENGOZIAN N. (1969) Marmosets: their potential in experimental medicine. *Ann. N.Y. Acad. Sci.* **162,** 336.

GENGOZIAN N., BATSON J.S. & EIDE P. (1964) Hematologic and cytogenetic evidence for hematopoietic chimerism in the marmoset, *Tamarinus nigricollis. Cytogenetics,* **3,** 384.

GENGOZIAN N., BATSON J.S., GREENE C.T. & GOSSLEE D.G. (1969) Hemopoietic chimerism in imported and laboratory-bred marmosets. *Transplantation,* **8,** 633.

GENGOZIAN N., BATSON J.S. & SMITH T.A. (1978) Breeding of marmosets in a colony environment. In: *Marmosets in Experimental Medicine.* Prim. Med. Vol. 10, p. 71, GENGOZIAN and DEINHARTD. Karger, Basel.

GENGOZIAN N., BAZZELL S.J. & NICKERSON D.A. (1976) Histocompatibility antigens and antibody forming cells in the marmoset, a natural blood chimera. *Fed. Proc.* **35,** 826 (abstract).

GENGOZIAN N. & PORTER R.P. (1971) Transplantation immunology in the marmoset. *Medical Primatology* (ed. by E. I. Goldsmith and J. Moor-Jankowski), p. 165. Karger, Basel.

GENGOZIAN N., SALTER B.L., BASFORD N.L. & KATELEY J.R. (1976) Characterization of the antibody response of the marmoset to sheep red blood cells. *Clin. exp. Immunol.* **23,** 525.

HARVEY J.S. JR., FELSBURG P.J., HEBERLING R.L., KNIKER W.T. & KALTER S.S. (1974) Immunological competence in non-human primates: differences observed in four species. *Clin. exp. Immunol.* **16,** 267.

HILL J.P. (1932) II. Croonian Lecture. The developmental history of the primates. *Phil. Trans. roy. Soc. London,* **221B,** 45.

HUMPHREY J.H., PARROTT D.M. & EAST J. (1964) Studies on globulin and antibody production in mice thymectomized at birth. *Immunology,* **7,** 419.

JACKSON A.L. & WALTERS C.C. (1972) Cells producing low-molecular weight antibody to *Escherichia coli* lipopolysaccharide. *Infection and Immunity,* **6,** 545.

JERNE N.K. & NORDIN A.A. (1963) Plaque formation in agar by single antibody-producing cells. *Science,* **140,** 405.

KATELEY J.R., NICKERSON D.A. & GENGOZIAN N. (1977) Blastogenic response of marmoset lymphocytes: cytokinetics and identification of responsive cells. *Immunology,* **33,** 653.

LUZZATI A.L., TAUSSIG M.J., MEO T. & PERNIS B. (1976) Induction of an antibody response in cultures of human peripheral blood lymphocytes. *J. exp. Med.* **144,** 573.

MISHELL R.I. & DUTTON R.W. (1967) Immunization of dissociated spleen cell cultures from normal mice. *J. exp. Med.* **26,** 423.

MÖLLER G. & MICHAEL G. (1971) Frequency of antigen-sensitive cells to thymus-independent antigens. *Cell. Immunol.* **2,** 309.

NIBLACK G.D. & GENGOZIAN N. (1976) T and B lymphocytes in the marmoset, a natural hemopoietic chimera. *Clin. exp. Immunol.* **25,** 536.

NICKERSON D.A. & GENGOZIAN N. (1976) Primary *in vitro* antibody formation by blood leucocytes of a sub-human primate. *Cell. Immunol.* **27,** 171.

NOSSAL G.J., ADA G.L. & AUSTIN C.M. (1964) Antigens in immunity. II. Immunogenic properties of flagella, polymerized flagellin and flagellin in the primary response. *Aus. J. exp. Biol. med. Sci.* **42,** 283.

PLOTZ P.H., TALAL N. & ASOFSKY R. (1968) Assignment of direct and facilitated hemolytic plaques in mice to specific immunoglobulin classes. *J. Immunol.* **100,** 744.

PORTER R.P. & GENGOZIAN N. (1969) Immunological tolerance and rejection of skin allografts in the marmoset. *Transplantation,* **8,** 653.

PORTER R.P. & GENGOZIAN N. (1973) Immunological responsiveness and tolerance of marmoset lymphoid tissue *in vitro. Transplantation,* **15,** 221.

ROWLEY M.J. & MACKAY I.R. (1969) Measurement of antibody-producing capacity in man. I. The normal response to flagellin from *Salmonella adelaide. Clin. exp. Immunol.* **5,** 407.

SHINOHARA N. & KERN M. (1976) Differentiation of lymphoid cells: B cell as a direct target and T cell as a regulator in lipopolysaccharide-enhanced induction of immunoglobulin production. *J. Immunol.* **116,** 1607.

URSO P. & GENGOZIAN N. (1973) T-cell deficiency in mouse allogeneic radiation chimeras. *J. Immunol.* **111,** 712.

URSO P. & GENGOZIAN N. (1974) Variation in T and B cell deficiency in different allogeneic radiation chimeras. *J. Immunol.* **113,** 1770.

WARNER C.M., FITZMAURICE M., MAURER P.H., MERRYMAN C.F. & SCHMERR M.J. (1973) The immune response of tetraparental mice to two synthetic amino acid polymers: 'high-conjugation' 2,4 dinitrophenyl-glutamic acid^{57}-lysine^{38}-alanine^{5} (DNP-GLA^{5}) and glutamic acid^{60} alanine^{30} tyrosine^{10} (GAT^{10}). *J. Immunol.* **111,** 1887.

WISLOCKI G.B. (1939) Observations on twinning in marmosets. *Am. J. Anat.* **64,** 445.