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The Transformation of Human Lymphocytes by Monkey Antisera to Human Immunoglobulins

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Summary. Cultures of human peripheral leucocytes were stimulated to incorporate tritiated thymidine when incubated with monkey antisera to human immunoglobulins. Twenty-five of forty-four monkey antisera were active and stimulated 90 per cent of leucocyte (WBC) cultures to incorporate a small but significantly greater amount of tritiated thymidine (TdR^3H) than that incorporated by controls. This stimulation of TdR^3H uptake correlated with an increase from 2 to 8 per cent lymphoblasts in the cultures. Leucocytes washed free of serum immunoglobulins responded to a greater degree to the anti-immunoglobulin sera than when they were cultured in the presence of human serum. Prior absorption of antisera with either whole serum or homologous immunoglobulin blocked antiserum stimulation completely. The anti-IgG and anti-IgM antisera were consistently more effective than anti-IgA, anti-κ and anti-λ chain antisera. Sequential stimulation by antisera against two different immunoglobulins was not significantly different from those stimulated by only one of the two. Lymphocytes from three asymptomatic subjects with low or absent serum IgA levels transformed as well with anti-IgA as did lymphocytes from subjects with normal serum IgA levels. Antisera were cytotoxic to the lymphocytes only in the presence of complement. Presumably the transformation of human lymphocytes was due to a reaction of anti-immunoglobulin antisera with specific immunoglobulin antigenic determinants present on or in the circulating lymphocytes.

INTRODUCTION

Sell and Gell (1965a, b) reported that rabbit anti-rabbit immunoglobulin allotype and sheep anti-rabbit immunoglobulin antisera stimulate rabbit lymphocytes to transform *in vitro*. They found the specificity of transformation with anti-allotype antisera to correlate with the genetic allotype of the lymphocyte. The ability of lymphocytes to respond was not related to the presence of serum immunoglobulins. Sheep anti-rabbit immunoglobulin antisera directed specifically against IgG or IgM each stimulated up to 80 per cent of the rabbit lymphocytes to transform, whereas anti IgA stimulated about 25 per cent (Sell, 1967). Unlike the recognition of the specific immunoglobulin allotype of the lymphocyte, the anti-immunoglobulin antisera apparently stimulated the same lymphocytes. This led Sell (1967) to suggest that each lymphocyte, although it expresses only one allotype, does contain the antigenic determinants of more than one immunoglobulin class at a time.

We have studied the effects of anti-immunoglobulin antisera on leucocyte cultures of

man because their immunoglobulins are well characterized, lymphocytes grow well, and subjects with low immunoglobulin levels are available for study. We have observed that some monkey anti-immunoglobulin antisera stimulate human lymphocytes (Oppenheim, Rogentine and Terry, 1967) and Adinolfi, Gardner, Gianelli and McGuire (1967) reported a horse anti-human H-chain serum with a similar degree of activity. We have found that about 56 per cent (twenty-five out of forty-four) of specific monkey antisera directed against various purified preparations of human immunoglobulins stimulated a small proportion of lymphocytes to transform and synthesize DNA *in vitro*. This study demonstrates that human serum immunoglobulins block this stimulatory effect. We also have investigated the relationship of this phenomenon to the cytotoxicity of the antisera, and the immunoglobulins produced by the lymphocyte donor.

METHODS

Preparation of antisera

Adult Rhesus monkeys were immunized with 5–10 mg of the purified immunoglobulins emulsified with complete Freund's adjuvant (CFA, Difco, Detroit, Michigan). They were boosted with a similar dose 3–5 weeks later, and again at 2–3 month intervals thereafter.

Normal serum IgG was prepared from commercially obtained pooled human serum immunoglobulin which was further purified by anion exchange chromatography on diethylaminoethyl-cellulose (DEAE) in a 0·01 M phosphate buffer, pH 8. IgA was prepared from sera containing IgA-myeloma protein by preparative block electrophoresis followed by chromatography on DEAE. IgM was obtained from Waldenströms macroglobulinaemic sera by block electrophoresis followed by filtration through Sephadex G-200. κ and λ proteins were isolated from urine containing Bence Jones proteins by ammonium sulphate precipitation followed by DEAE chromatography and where indicated, Sephadex G-200 filtration. All antigens were assessed for purity by immunochemical means.

The monkey sera obtained after each antigen stimulation were tested for the presence of specific precipitating antibodies by immunodiffusion. Those antisera containing precipitating antibodies after the first to fourth booster doses of antigens were screened for *in vitro* lymphocyte stimulating activity. Only those with the greatest activity were used for extensive studies. All of these antisera were specific for the immunizing class of immunoglobulin except for the anti IgG which also reacted with κ-chains. None of the antisera reacted with non-immunoglobulin serum proteins, when tested by gel precipitation techniques. The relationship between appearance of lymphocyte-stimulating activity and immunization course varied from animal to animal. Control monkey sera were obtained from three different unimmunized monkeys, one of which had active tuberculosis. All sera were filter sterilized (Nalgene 0·2 μ diameter filters, Nalge Co., Inc., Rochester, New York), and heat inactivated at 56° for 30 minutes.

Preparation of cell suspension

Peripheral leucocytes from normal volunteers were obtained by sedimentation of the RBC in 120 ml of heparinized (20–30 u/ml) blood. After 1–2 hours at 37°, the supernatant WBC-rich plasma was removed. The WBC were then centrifuged at 500 ***g*** (at room temperature) and the autologous plasma discarded. The WBC were then washed twice with Hanks's medium containing 5 per cent heat inactivated (56° for 30 minutes) agammaglobulinaemic calf serum, and resuspended in minimal essential medium (MEM) with

20 per cent agammaglobulinaemic calf serum. The MEM contained 2 mM glutamine, 50 units penicillin and 50 μg streptomycin/ml. This processing generally resulted in a relative increase in the percentage of lymphocytes present in the cell-suspension from a median of 50 to 60 per cent. The volume of cell suspension was then adjusted to a cell concentration of $2{\cdot}4 \times 10^6$/ml. This was divided into 2·5 ml aliquots in plastic tissue culture tubes (Falcon Plastics, Los Angeles, California, 16×125 mm No. 3033).

Culture conditions

The 2·5 ml control cell suspensions were cultured in duplicate with 0·5 ml serum from immunized monkeys (diluted 1:8 with MEM) or 0·5 ml MEM only. Other 2·5-ml aliquots obtained from the same subject were cultured in duplicate with 0·5 ml of a 1:8 dilution of antiserum in MEM. Cell suspensions with a total volume of 3 ml were then incubated for 3–5 days at 37° in an atmosphere of 5 per cent CO_2 and 95 per cent air.

Harvesting

Two micro-curies tritiated thymidine (TdR ^{3}H, specific activity 6·7 c/mM, New England Nuclear) were added 4 hours prior to termination of the culture period. Samples (0·5 ml) from representative cultures were used to prepare Giemsa stained smears. Four hundred cells per coded slide were analysed to determine the per cent of intact mononuclear cells which were transformed lymphocytes. The remaining cell suspension was washed with cold isotonic saline, and trichloroacetic acid precipitable counts determined by methods as previously indicated (Oppenheim, Wolstencroft and Gell, 1967).

Cytotoxicity assays

The monkey antisera were tested for complement dependent cytotoxicity against human peripheral blood lymphocytes by the ^{51}Cr release cytotoxicity technique (Rogentine and Plocinik, 1967).

Statistical methods

The TdR ^{3}H uptake results were distributed in such a way that a logarithmic transformation yielded normally distributed data. The geometric means and Student's *t*-tests of paired comparisons of such log transformed data were determined with the aid of a computer program.

RESULTS

Preliminary studies indicated that human leucocyte cultures containing 1:6 dilutions of monkey anti-human IgG or IgM showed increased uptake of TdR ^{3}H when compared with controls containing 1 : 6 dilutions of unimmunized monkey serum. This stimulation persisted with further serial two-fold dilution of the antisera to 1:96, but diminished thereafter. In most studies the antisera were used at dilutions of 1:48.

The leucocytes stimulated with anti-immunoglobulin incorporated more TdR ^{3}H when cultured with agammaglobulinaemic calf serum instead of autologous human plasma. Fig. 1 compares the ratios of TdR ^{3}H uptake by cultures stimulated with anti-IgG and anti-IgM to that of control cultures which were incubated either in autologous plasma or calf serum. The *in vitro* proliferation of leucocytes stimulated with anti-immunoglobulin antisera was significantly greater when the leucocytes were washed twice and cultured in

MEM with 16·7 per cent heat-inactivated agammaglobulinaemic calf serum than when they were washed twice and cultured in MEM containing 16·7 per cent autologous plasma. However, despite the presence of IgG in the autologous plasma the anti-IgG still stimulated an increased TdR^{3}H uptake over that by control cultures in autologous plasma ($P<0·025$). In all subsequent studies the leucocytes were washed free of human plasma and were incubated in the presence of calf serum.

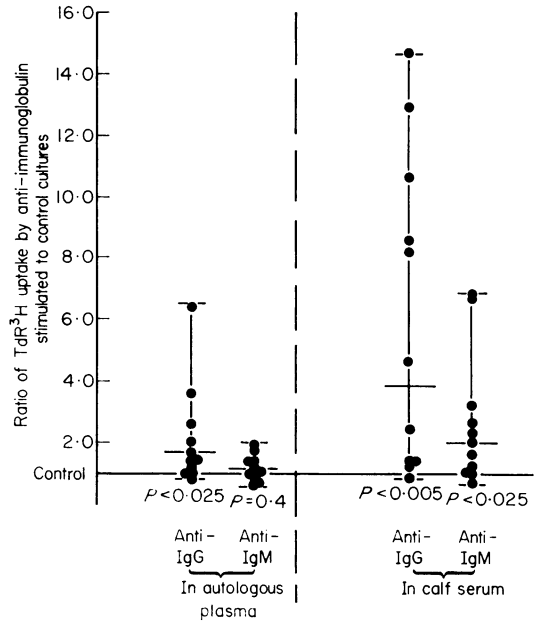

FIG. 1. The geometric mean and range of ratios of tritiated thymidine incorporation by anti-IgG or anti-IgM stimulated leucocyte cultures to that of control cultures containing unimmunized monkey serum. The leucocytes were washed and cultured with MEM, containing either 16·7 per cent autologous plasma or agammaglobulinaemic calf serum.

The lymphocyte proliferation stimulated by anti-immunoglobulin was evident by 68 hours of incubation but was greater by 92 and 116 hours (Fig. 2). With longer periods of incubation both the uptake of tritiated thymidine and proportions of transformed lymphocytes were increased. Subsequent cultures were, therefore, routinely incubated for five days. In this period of time the increments in the lymphocyte transformation stimulated by anti-immunoglobulin were usually greater than any stimulation by the unimmunized monkey serum, calf serum (Shrek and Elrod, 1964) or other antigenic components of the media (Johnson and Russell, 1965).

The effect of duration of exposure of the leucocytes to an antiserum was studied by exposing them for progressively longer periods of time to either anti-IgG or anti-IgM (Fig. 3). Leucocyte suspensions were incubated for 20 minutes at 37°. Antisera were added for progressively increasing periods of 5–340 minutes. Thereafter the cultures were centrifuged, 500 ***g*** at room temperature for 10 minutes, washed once and cultured for 5 days in medium without any additional antiserum. The TdR^{3}H incorporation of cultures treated this way was expressed as a per cent of the TdR^{3}H uptake by cultures incubated

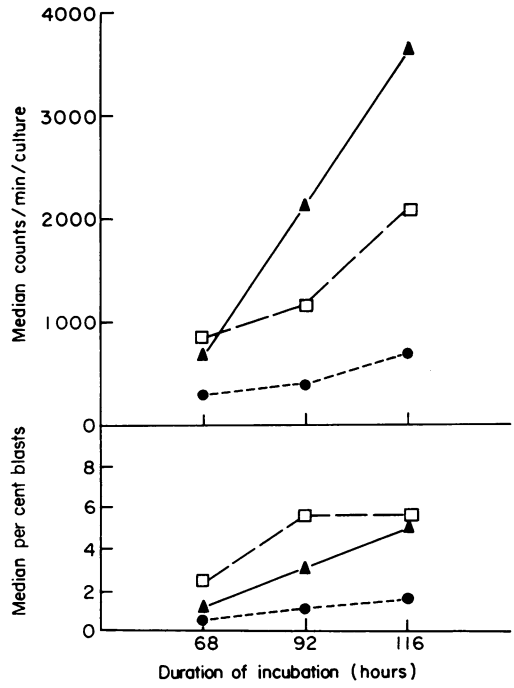

FIG. 2. The median per cent lymphoblasts and TdR^{3}H incorporation by leucocytes cultured for 68–116 hours with: (▲) anti-IgG, (□) anti-IgM or (●) unimmunized monkey serum.

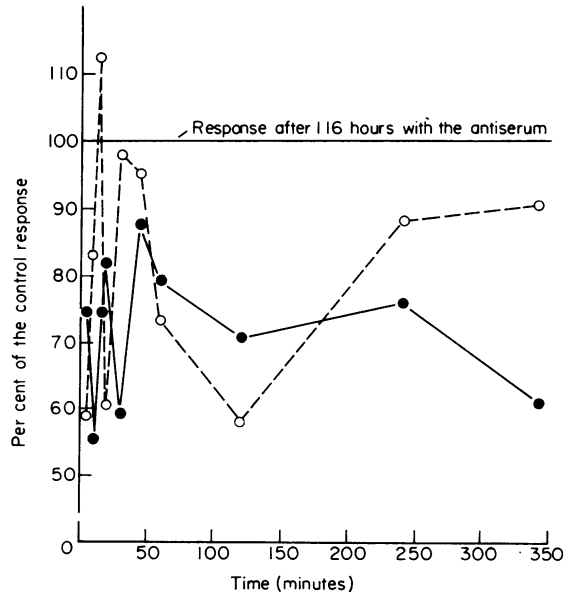

FIG. 3. The TdR^{3}H incorporation by leucocyte cultures pulsed for 5–350 minute with: (●) anti-IgG or (○) anti-IgM serum. The results are expressed as a percentage of TdR^{3}H incorporation by leucocyte cultures exposed to the anti-IgG or anti-IgM for the entire 5-day period of incubation.

with the antisera for 25 minutes, centrifuged, washed, and *re-exposed* to the antiserum for the remainder of the 5-day period (Fig. 3). The leucocyte cultures which were thus exposed for only 5–340 minutes at 37° to the antisera showed from 55 to 112 per cent of the activity of cultures exposed to the antisera for the entire 120 hours of incubation. This suggests that the interaction of antisera with the lymphocytes occurs almost instantaneously.

The response of leucocytes cultured for 5 days at cell concentrations ranging from 1 to 7×10^6/ml was determined (Fig. 4). This in effect tested the efficacy of lymphocyte proliferation in tubes with cell cultures containing from 3 to 21×10^6 leucocytes. The optimal ratio of TdR^{3}H uptake by anti-IgG stimulated to that of unstimulated cultures was at 2×10^6 leucocytes/ml of cell suspension. Subsequent cultures were, therefore, all done at this concentration.

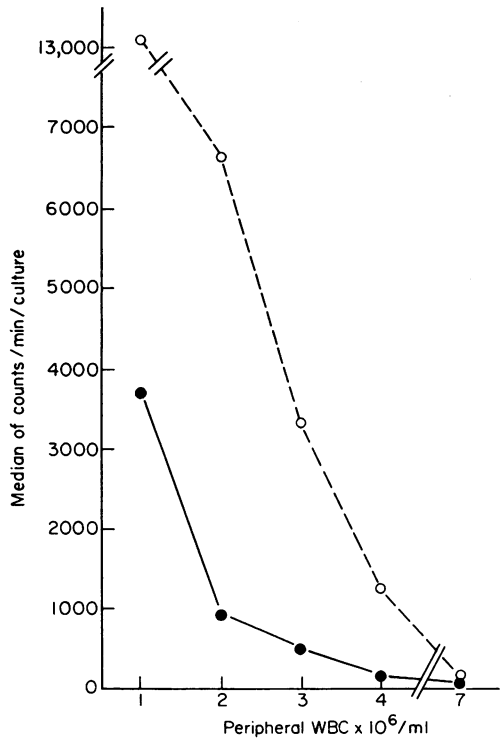

FIG. 4. The effect of increasing concentration of WBC on the median TdR^{3}H incorporation by (●) unstimulated and (○) anti-IgG stimulated leucocyte cultures.

Twenty-five of forty-four antisera obtained from fifteen monkeys actively stimulated human lymphocyte proliferation. IgG, IgM, IgA, κ or λ Bence Jones proteins were each used to immunize from two to five different monkeys. Their antisera were obtained and tested. Only the results achieved with the most active antisera will be reported (Fig. 5). The ratio of the TdR^{3}H uptake in unstimulated (calf serum only) or various antisera stimulated cultures to that of cultures incubated with sera from one of three unimmunized monkeys (control) is depicted. On more than 90 per cent of occasions (116 of 127) these

active antisera stimulated cultures from different subjects to incorporate more TdR^3H than was incorporated by control cultures. The TdR^3H uptake by cultures containing only calf serum did not differ significantly from those with added unimmunized monkey serum (controls) ($P = 0{\cdot}6$). The anti-IgG obtained from a monkey immunized with pooled normal human IgG was considerably more active than that from two monkeys that had been immunized with the IgG from single donors. This was so even when the WBC from those particular IgG donors themselves were tested. It was also more active than three

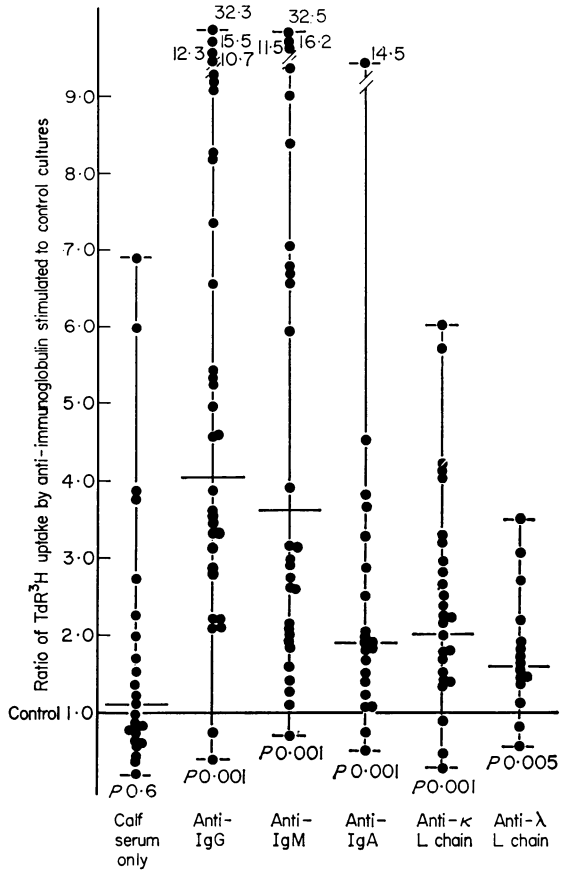

FIG. 5. The geometric mean and range of ratios of TdR^3H incorporation by leucocytes with added calf serum only, anti-IgG, anti-IgM, anti-IgA, anti-κ or anti-λ chain antisera to that of control cultures containing only unimmunized monkey and calf serum.

monkey anti-myeloma IgG antisera. The most active anti-IgM antiserum was obtained from one of two monkeys immunized with a myeloma M protein. Similarly the most active anti-IgA was one of two antisera from monkeys immunized with a myeloma IgA. Only one of two antisera prepared against κ or λ Bence Jones proteins was active. The anti-IgG and anti-IgM antisera were consistently more active stimulants of human lymphocytes than the anti-IgA, anti-κ and anti-λ antisera.

In four studies the per cent lymphoblasts appearing in antisera-stimulated cultures were determined (Fig. 6). The increments in the per cent transformed lymphocytes in the antisera-stimulated cultures over the median of 2 per cent lymphoblasts present in the control cultures (with and without unimmunized monkey serum) were small but statistically significant. As with the TdR ^{3}H uptake determinations the anti-IgG and anti-IgM were

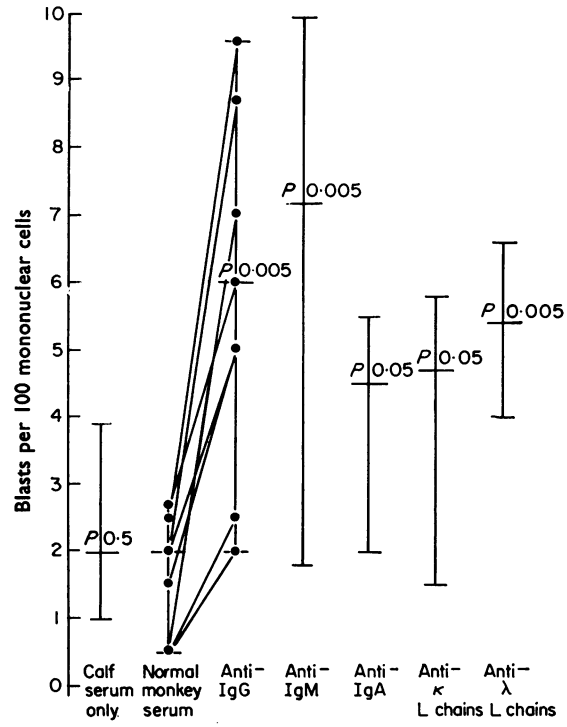

FIG. 6. The median and range of per cent lymphoblasts per 100 mononuclear cells appearing in leucocyte cultures which contained only calf serum, calf serum and unimmunized monkey serum, or anti-IgG, anti-IgM, anti-IgA, anti-κ or anti-λ chain antisera are shown. The response of cultures containing unimmunized monkey serum, and response of the corresponding anti-IgG stimulated cultures are shown by connecting lines.

most effective and stimulated a median of 6 and 7 per cent lymphocytes to transform. The anti-IgA, κ and λ antisera were less effective and stimulated a median of 4·5, 4·7 and 5·4 per cent lymphocytes, respectively, to transform.

To reassure ourselves that we were, indeed detecting lymphocyte stimulation by antisera directed specifically against the three classes of immunoglobulins the antisera were absorbed with the other immunoglobulin antigens. The anti-IgG was absorbed with pooled normal κ and λ chains; the anti-IgM was absorbed with pooled human IgG and agammaglobulinaemic serum; and the anti-IgA with IgA deficient whole human serum. The degree of lymphocyte stimulation by these 'specific' antisera was then compared with that of their unabsorbed counterparts (Fig. 7). There were no differences except for a significant improvement in the stimulation by the anti-IgA after absorption ($P = 0{\cdot}025$).

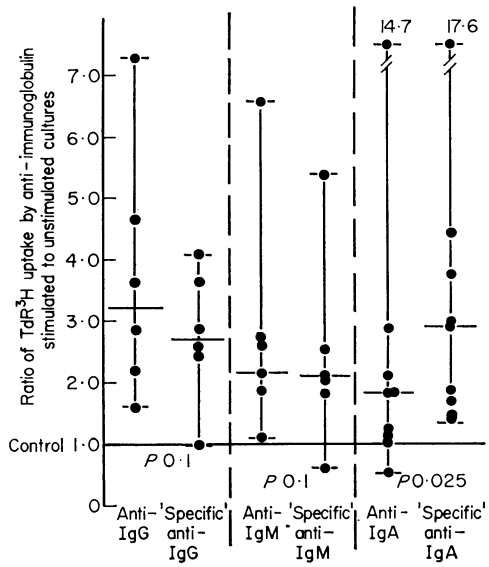

FIG. 7. A comparison of the geometric mean and range of ratios of TdR^3H uptake by unabsorbed and absorbed 'specific' anti-IgG, anti-IgM, and anti-IgA stimulated leucocyte cultures to control cultures containing unimmunized monkey serum. The anti-IgG was absorbed with pooled κ and λ chains; the anti-IgM with pooled human IgG; and the anti-IgA with IgA-free whole human serum.

The antisera in addition to stimulating some lymphocytes to transform were also associated with morphological evidence of cytotoxicity to the lymphocytes such as an increased incidence of karrhyorhexis, karyolysis and pyknosis. The cytotoxic properties of the antisera were, therefore, tested for complement dependent cytotoxicity by the technique of ^{51}Cr release of Rogentine and Plocinik (1967) (see Table 1). The lymphocytes

TABLE 1

CYTOTOXICITY OF MONKEY ANTISERA*

	Percentage maximal ^{51}Cr release	
	Lymphocyte donor No. 1	Lymphocyte donor No. 2
Anti-IgG	28	29
Anti-IgM†	25	33
Anti-IgA before absorption	45	44
Anti-IgA absorbed with IgA deficient serum	14	17
Anti-κ L chain†	15	11
Anti-λ L chain†	39	41
Control monkey sera‡	38	46
Normal human serum and complement†§	5	8
Buffer and complement†	8	9
Buffer without complement†	4	5
Antiserum without complement	<7	<7

* Positive results are per cent ^{51}Cr release $\geqslant 15$ per cent above NHS control. Negative results are per cent ^{51}Cr release $\leqslant 7$ per cent above NHS control.

† Average result of the two different sera used or test done twice.

‡ Average result of three different sera used.

§ Complement source was rabbit serum diluted 1 : 2.

from the two test subjects were also successfully transformed with all the active antisera. All the antisera except those against κ chains showed complement dependent cytotoxicity, which however was no greater than that of control monkey sera. The anti-IgA became both significantly less cytotoxic and more stimulating after absorption (Fig. 7) suggesting that cytotoxic antibodies had been removed and were not related to the antibodies responsible for transformation. In the absence of complement none of the monkey antisera released more ^{51}Cr than control normal human serum. Although the cultured leucocytes were washed and the monkey and calf sera with which they were cultured were heat inactivated, the cytotoxicity of the antisera still may have been due to adherence of heat labile complement components to the surface of the leucocytes.

Since antisera directed against different immunoglobulins were capable of stimulating lymphocytes it was important to determine whether they were stimulating the same or different populations of lymphocytes. This was investigated by adding two different anti-immunoglobulin antisera sequentially to the cultures. The cells were incubated for 2–16 hours with a 1:96 dilution of the first antiserum at 37°, washed once with Hanks's solution containing 5 per cent agammaglobulinaemic calf serum, resuspended in fresh medium and restimulated with the same or a different antiserum (1:96). Control cultures with serum from unimmunized monkeys were similarly incubated, washed and re-exposed to the serum. Such sequentially stimulated cultures incorporated significantly more TdR^3H ($P < 0.01$) than control cultures (Fig. 8). There was only a small insignificant increase in the response of cultures stimulated sequentially with anti-IgG plus anti-IgM in comparison

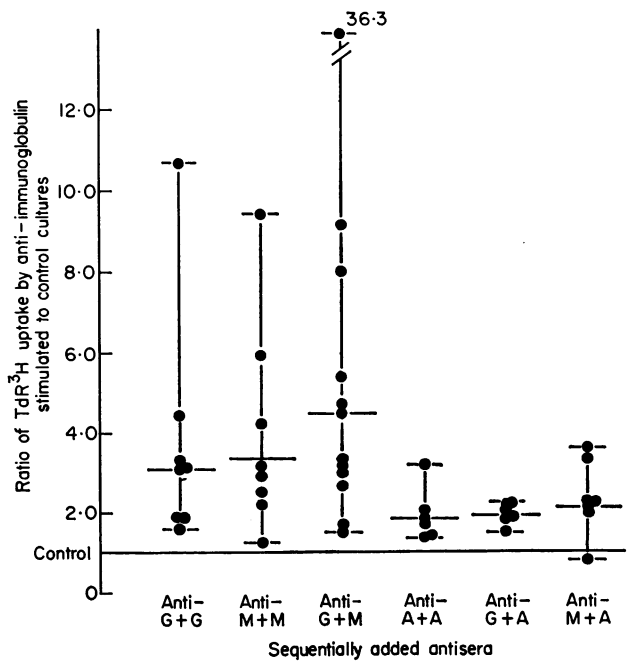

FIG. 8. The geometric mean and range of ratios of TdR^3H incorporation of leucocyte cultures stimulated sequentially by 1:96 dilutions of anti-IgG and anti-IgG, anti-IgM and anti-IgM, anti-IgG and anti-IgM, anti-IgA and anti-IgA, anti-IgG and anti-IgA, or anti-IgM and anti-IgA to that of control cultures to which unimmunized monkey serum had been added twice.

with cultures stimulated with anti-IgG plus anti-IgG or anti-IgM plus anti-IgM ($P = 0{\cdot}5$ and 0·4, respectively). There was a moderate insignificant decrease in the response of anti-IgG plus anti-IgA and anti-IgM plus anti-IgA stimulated cultures compared with those stimulated either with anti-IgG plus anti-IgG or anti-IgM plus anti-IgM ($P < 0{\cdot}2$ and 0·4, respectively).

The effect of prior absorption with whole human serum on the stimulatory activity of the antisera was investigated. Both anti-IgG and anti-IgM were incubated for 48 hours at 4° with heat inactivated normal human serum (NHS). The stimulation by the anti-IgG was inhibited by as little as an equal volume of a 1:1000 dilution of the serum, but more effectively by an equal volume of undiluted NHS (Fig. 9). Neither the washed precipitates of anti-IgG–IgG nor the supernatant resulting from such an absorption contained

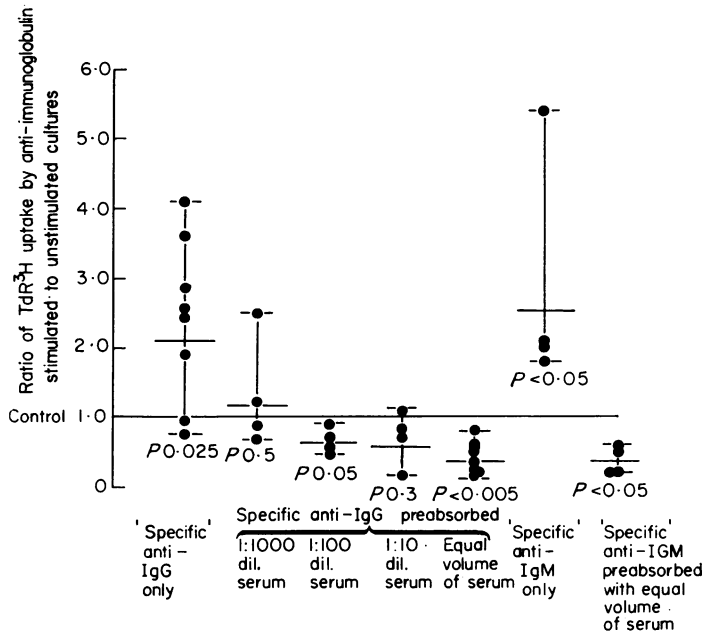

FIG. 9. The geometric mean and range of ratios of TdR³H incorporation by anti-IgG and IgM stimulated leucocyte cultures before and after absorption of the antisera for 48 hours with heat inactivated whole serum to that of controls containing unimmunized monkey serum.

any residual stimulating activity. In fact after absorption with an exceess of antigen both the absorbed anti-IgG and anti-IgM antisera inhibited the TdR³H uptake by the human lymphocytes to levels significantly below that of control culture ($P < 0{\cdot}005$ and $< 0{\cdot}01$, respectively). Inspection of slides from such inhibited cultures did not reveal any increase in cytotoxicity. The small lymphocytes remained intact as well as untransformed.

Using a similar approach, the stimulatory effect of 1 ml of anti-κ chain antisera was blocked by prior absorption with increasing quantities of 10, 100 and 400 μg κ Bence Jones proteins (Fig. 10). It was partially blocked by prior absorption with λ Bence Jones protein (P diminished to $< 0{\cdot}1$ in comparison with control). We do not know whether this reduction may have been due to a minimal contamination of the λ with κ chains, or

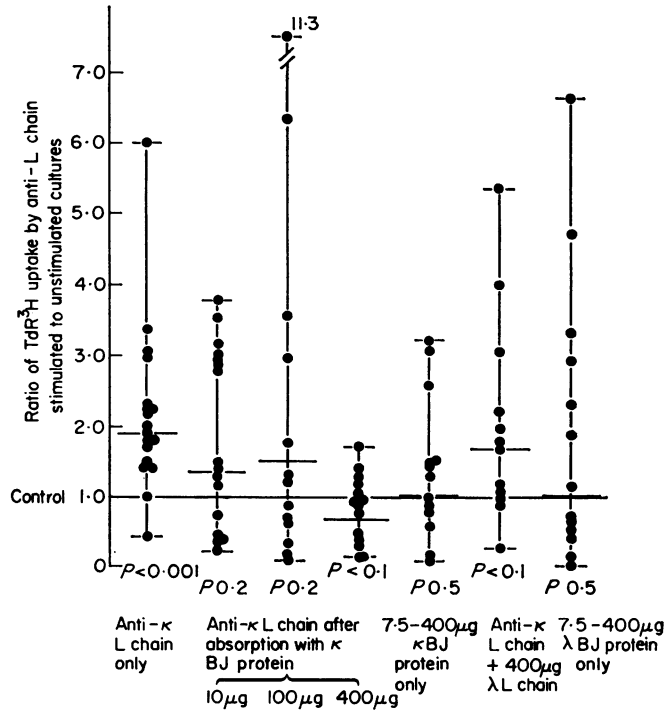

FIG. 10. The geometric mean and range of ratios of TdR³H incorporated by leucocyte cultures stimulated by anti-κ chain antiserum before and absorption with κ and λ chain antigen. The effects of κ and λ L chains by themselves are also shown.

was due to some cross-reactivity between the λ and κ chains. In any case the κ chains were considerably more effective in blocking the activity of the antiserum than the λ chains ($P < 0{\cdot}001$). Neither the κ nor λ chains by themselves stimulated lymphocyte transformation.

The ability of anti-IgA to transform lymphocytes from subjects deficient in serum IgA was tested. Three normal volunteers with low serum IgA were tested. These normal volunteers had serum IgA concentration of 0·7 mg/ml 0·21 mg/ml, and less than 0·06 mg/ml (Fig. 11). Their lymphocytes were stimulated significantly by the anti-IgA ($P < 0{\cdot}005$). The degree of response of the IgA deficient subjects was not significantly different from that of controls having normal levels of $3{\cdot}9 \pm 0{\cdot}9$ mg/ml serum IgA (χ^2 $P = 0{\cdot}5$). The lymphocytes from several of the latter subjects failed to respond to anti-IgA when incubated in their own IgA rich plasma, whereas the lymphocytes from several IgA deficient subjects proliferated just as well in the presence of their IgA deficient plasma as in the calf serum.

DISCUSSION

Presumably the ability of immunospecific monkey antisera directed against a variety of serum immunoglobulins to stimulate a low proportion of human lymphocytes to proliferate *in vitro*, reflects a reaction of the antiserum with antigens common to both serum

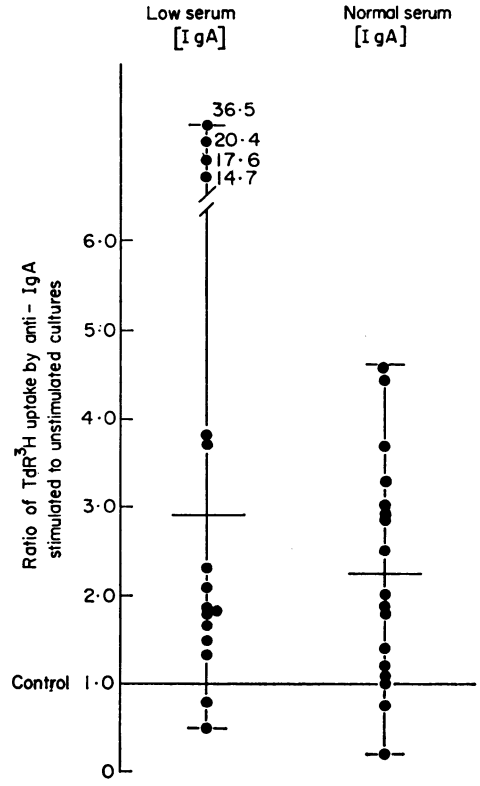

FIG. 11. A comparison of the geometric mean and range of ratios of TdR^3H incorporation by anti-IgA stimulated leucocyte cultures to the response of control cultures from subjects with low or absent serum IgA to those having normal serum IgA levels.

immunoglobulins and the cell surface. The stimulatory effects of the monkey antisera persisted even after absorption with all but the specific immunoglobulin against which they were directed. The enhanced thymidine incorporation of leucocyte cultures by anti-IgG was not significantly reduced after absorbing it with pooled κ and λ chains. The activity of the anti-IgM was not reduced by absorption with pooled human IgG. Absorption of the anti-IgA with IgA-deficient human serum increased its stimulatory effect significantly with a concomitant reduction of its cytotoxic effects.

The stimulatory effects of the anti-immunoglobulin antisera were blocked by the specific immunoglobulin against which they were directed. The relatively weak stimulation of lymphocyte transformation by anti-κ antiserum was readily blocked by prior absorption with an excess of Bence Jones protein. The λ chains were significantly less efficacious in blocking the lymphocyte stimulation by the anti-κ serum. The lymphocyte stimulating activity of the anti-IgG and anti-IgM antisera was also blocked by prior absorption with normal whole human serum. The resulting antigen-excess complexes of anti-IgG and anti-IgM produced a significant and apparently non-toxic inhibition of the low grade stimulation of human lymphocytes by calf serum and other media components. This inhibitory effect of immunoglobulin–anti-immunoglobulin complexes is in contrast to the report of stimulation of previously unsensitized lymphocytes by complexes of antigens and

antibodies (Bloch-Schtacher, Hirshhorn and Uhr, 1967). Subsequent studies in this laboratory indicate that soluble complexes in antigen excess cause this inhibition, and that they also inhibit the transformation of human lymphocytes with various other specific antigenic as well as 'non-specific' stimuli. These observations preclude the use of inhibition of transformation by these absorbed antisera as an argument for specificity.

The presence of human serum immunoglobulins interfered with the ability of lymphocytes to be transformed by anti-immunoglobulin antisera. Optimal stimulation was obtained with lymphocytes washed free of human serum and cultured in medium with agammaglobulinaemic calf serum. Only the anti-IgG, although reduced in activity, was still significantly stimulatory in the presence of an excess of serum IgG in the form of autologous plasma (Fig. 1) suggesting that the antibody was not immediately irreversibly bound to the serum IgG.

Passively adsorbed immunoglobulins are unlikely to be responsible for the anti-immunoglobulin stimulated lymphocyte transformation since the addition of preformed complexes such as IgG–anti-IgG in antigen excess inhibited rather than stimulated the lymphocytes (Figs. 9 and 10). Furthermore, the transformation of lymphocytes by anti-IgA was not diminished in the presence of IgA-deficient human plasma, whereas it was blocked by whole human plasma. This is consistent with the data of Sell and Gell (1965a, b) who also demonstrated antisera stimulation to be independent of serum immunoglobulins. However, we can not rule out the possibility that immunoglobulins may be irreversibly adsorbed on receptor sites of the lymphocyte.

The anti-IgG and anti-IgM antisera consistently stimulated significantly more lymphocyte transformation than the most active anti-IgA, anti-κ and anti-λ antisera. Similarly Sell (1967) also consistently observed sheep anti-rabbit IgG and IgM to be more active than anti-IgA. This may reflect a greater representation of the IgG and IgM antigenic configurations on the cell surface of the lymphocyte population, or alternatively reflects the fact that the IgA, κ and λ antigens did not elicit as effective a precipitin antibody response as the IgG and IgM in our studies.

We found that only a small proportion of less than 10 per cent of human lymphocytes were transformed by monkey antisera as did Adinolfi *et al.* (1966) with horse anti-H chain serum. This contrasts sharply with the much greater transformation of up to 80 per cent of rabbit lymphocytes by sheep anti-rabbit immunoglobulin antisera (Sell, 1967). It has been observed that PHA-stimulated lymphocyte transformation and delayed hypersensitivity are less active in the rabbit than in man (Knight, Ling, Sell and Oxnard, 1965). It has been reported that the lymphocytes of bursectomized chickens cannot be stimulated with anti-immunoglobulin antisera (Peterson, personal communication 1968) while lymphocytes from thymectomized rats do not transform as well with PHA (Rieke, 1966). It is therefore conceivable that the greater reaction to anti-immunoglobulins, and lower efficacy of PHA stimulated peripheral lymphocyte transformation by the rabbit may be due to the presence of greater proportions of antibody producing than 'thymus derived' lymphocytes in the peripheral circulation of the rabbit than in man. Alternatively, the greater effect of sheep antisera on rabbit than monkey antisera on human lymphocytes may be related to the greater phylogenetic differences between sheep and rabbit, than between monkey and man.

Cultures stimulated by two antisera directed against two different immunoglobulins did not manifest significantly more lymphocyte proliferation than those stimulated by only one of those two antisera. This differs from the observed summation of *in vitro* stimulation

by antisera to different rabbit allotypes, which was interpreted to indicate that the antisera specifically detect rabbit lymphocytes that produce one or the other of the allotypes (Gell and Sell, 1965). The sequential stimulation of human lymphocytes by anti-IgG and then anti-IgM was only minimally additive, and when cultures were stimulated by anti-IgG or anti-IgM followed by anti-IgA the lymphocyte transformation was less than that stimulated by anti-IgG or IgM by themselves. These findings and similar observations of Sell (1967) with sheep anti-rabbit immunoglobulin antisera favour the view that the same lymphocytes may be responding to both the anti-IgG and anti-IgM antisera. The failure to obtain summation provides an argument for the view that single cells contain antigens of more than one class of immunoglobulin. However, this argument is weak since the sequential stimulation of lymphocytes by two or more antigens also fails to produce summative effects (Caron, 1967). This occurs despite the observation that antigens stimulate different populations *in vitro* (Dutton and Mishell, 1967). It is also possible that the antisera may be stimulating a small proportion of 'antiserum sensitive' cells to proliferate which then recruit the remainder from a common population.

We have used two additional approaches to investigate the relationship of immunoglobulin production to transformation of lymphocytes by anti-immunoglobulin antisera. The ability of lymphocytes from 'normal' subjects with low or absent serum IgA levels to respond normally to anti-IgA suggests that this phenomenon is not directly related to the secretion of normal quantities of IgA by the lymphocytes. No information is available about the IgA content in other body fluids of these subjects. They presumably have produced at least enough IgA-like antigen which is present on the lymphocyte surface and with which the specific anti-IgA reacts so as to trigger proliferation of a normal number of their lymphocytes. This suggests that the deficit in our subjects was merely quantitative. Furthermore, radioimmunoelectrophoretic studies reveal only minimal synthesis of IgG by occasional anti-immunoglobulin stimulated leucocyte cultures. This degree of immunoglobulin production was seen in a few anti-IgM, anti-IgA, and anti-IgG stimulated as well as control cultures (Asofsky, unpublished observations 1967). Thus, the transformation of lymphocytes by anti-immunoglobulin antisera perhaps detects only those cells capable of producing them, although not necessarily in quantities sufficient for export.

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