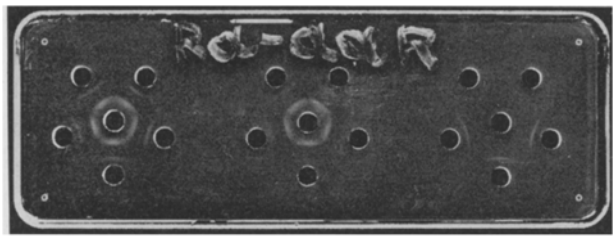

Fig. 4. Rheumatoid factor positive serum and rabbit anti-rheumatoid factor serum (Pentagone 1); normal human serum and rabbit anti-rheumatoid factor serum (Pentagone 2); and rheumatoid factor positive serum (Pentagone 3, lower wells) and normal human serum (Pentagone 3, upper wells) with absorbed rabbit anti-rheumatoid factor serum.

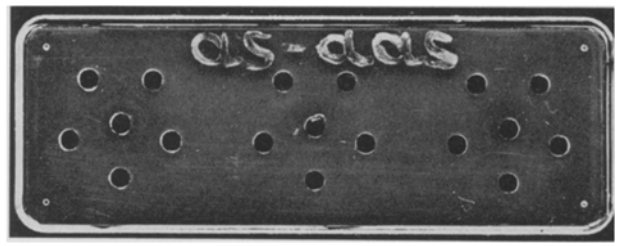

Fig. 5. Human anti-streptolysin 0 antibody with rabbit anti-anti-streptolysin serum (Pentagone 1); human anti-streptolysin 0 antibody (Pentagone 2, 3, lower wells) and normal human serum (Pentagone 2, 3, upper wells) with absorbed rabbit anti-anti-streptolysin 0 serum.

to precipitate the rheumatoid factor positive serum but failed to do so with normal serum (Figure 4).

Only 6 of the 9 rabbits injected with anti-streptolysin 0 produced precipitin antibodies. Their specificity was demonstrated after absorption of rabbit sera with normal human serum (Figure 5).

Discussion. This second report on the production of anti-antibodies by the 'immunological triangle' was intended to demonstrate the presence of specific anti-antibodies to relatively simple antigens such as albumin, streptolysin 0, Rh antigen and rheumatoid factor and confirms our previous work[8].

The results obtained indicate that we might be able to train the lymphopoietic system to produce specific anti-antibodies.

Résumé. Les anti-anticorps pourraient avoir des applications thérapeutiques dans certains états allergiques, les maladies d'auto-immunisation et dans les greffes des tissus. Les auteurs montrent la possibilité de stimuler la formation des anti-anticorps specifiques contre les anticorps produits par des antigènes relativement simples comme l'albumine, le facteur Rh, le facteur rheumatoïde et la streptolysine 0.

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The Growth of Tumour Allografts as a Measure of the Immunosuppressive Potency of Antilymphocyte Sera

The effects of antilymphocyte serum (ALS) on the immune response have been studied extensively and several methods for assessing the potency of different batches have been devised. There is however a need to evaluate all of the methods which may be utilised to assess the immunosuppressive potency of ALS in order to facilitate the development of satisfactory assay techniques which require minimal technical expertise and laboratory facilities.

The ability of ALS to prolong the survival of skin allografts has been exploited in methods for assaying the immunosuppressive potency of samples in vivo[1-3]. Alternative in vivo methods have utilised suppression of the production of plaque forming cells in response to sheep red blood cells[4,5], variations in the distribution of Cr^{51} labelled thymocytes between the liver and the spleen[5] and suppression of xenogeneic graft-versus-host reaction[6].

Initially attempts to assay ALS using in vitro methods were unsuccessful[7,8]. Recently however rosette inhibition[9], opsonization titre[10] and lymphocytophilic antibody titre[11] have been shown to correlate well with the immunosuppressive potency of ALS.

Tumour rejection is inhibited by ALS therapy[12-14] and the ability of ALS to facilitate tumour growth has been utilised to differentiate between active and inactive sera[15-19]. The possibility of exploiting measured changes in the growth rates of tumour allografts to assay the immunosuppressive potency of antilymphocyte sera has not however been explored.

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Materials and methods. The growth of subcutaneous implants of a CBA mammary adenocarcinoma has been followed by palpation and comparison with graded sized steel spheres. The origin and histology of the tumour have been described fully elsewhere together with the methods of transplantation and growth measurement[14, 20].

Antilymphocyte sera were produced by the 2 pulse-method of LEVEY and MEDAWAR[3]. Further batches of serum which were independently produced and tested for their immunosuppressive potency by determination of their ability to prolong the survival of skin allografts were supplied through the kindness of Dr. B. BRADLEY[21] and Dr. D. ARKELL[22].

A group of 5 female mice (CS1/Ash) weighing between 20 and 25 g was used to test each sample of serum. Following the implantation of a tumour allograft each animal was treated with 4 s.c. injections of 0.5 ml of ALS or normal rabbit serum (NRS) on days 0, 2, 4 and 6. The growth of the allografts was followed and their diameter was recorded regularly (1/16 inch units). Sera were randomized before use and scored blind.

Results. Although tumour allografts grew rapidly in some of the groups, the growth rates of the allografts varied and some of the sera failed to enable progressive growth to occur (Figure 1). The growth of tumour allografts was therefore followed in groups of mice treated with sera which had been assayed by measuring their effect on the survival of skin allografts.

The growth rates of tumour allografts were again accelerated to varying degrees (Figure 2). The mean diameter of tumour allografts on the 14th day after implantation was related to the corresponding mean survival time of skin allografts. There was a systematic error between the mean survival time of skin allografts estimated by the 2 investigators who provided samples of ALS. If however their results are normalized by calculating the percentage increase in skin allograft survival effected by ALS (Table), a good relationship between this value and the mean diameter of tumour allografts (1/16 inch units) on day 14 (D) can be demonstrated:

$$D = 0.11\,P + 3.21 \ldots 1. \quad (r = 0.95,\ p < 0.001)$$

$$\text{where } P = \left[\frac{\text{mean skin allograft survival time for the ALS}}{\text{mean skin allograft survival time for the NRS}}\right.$$

$$\left.\frac{\text{treated group}}{\text{treated group}} - 1\right] \times 100 \text{ (Figure 3).}$$

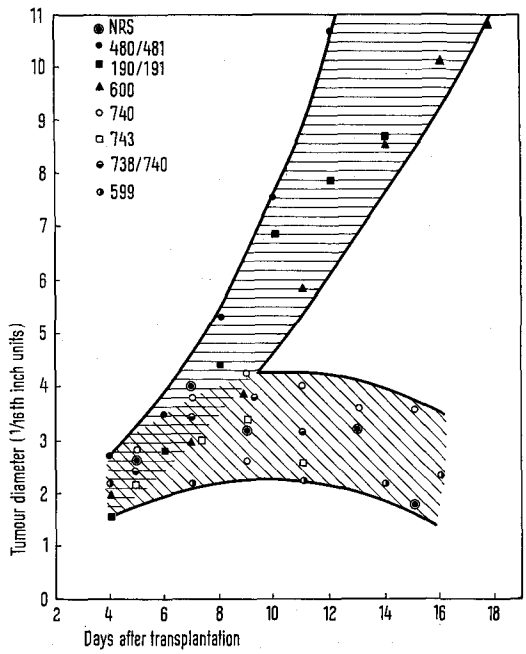

Fig. 1. The growth of tumour allografts in mice treated with different batches of ALS.

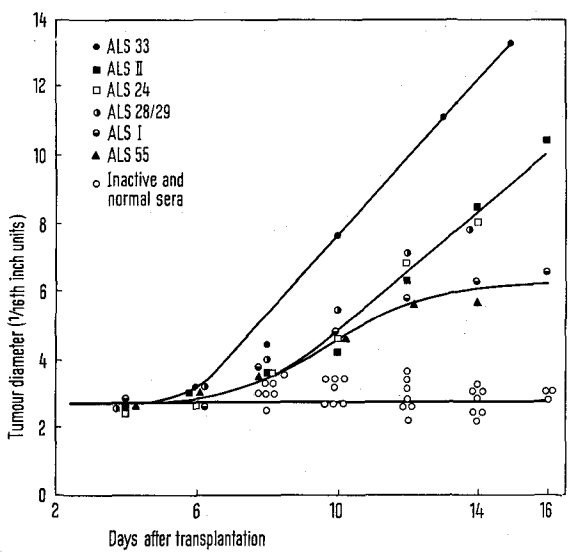

Fig. 2. The growth rates of tumour allografts in mice treated with various batches of ALS which had been independently assayed[21, 22] by determination of their ability to prolong the survival of skin allografts.

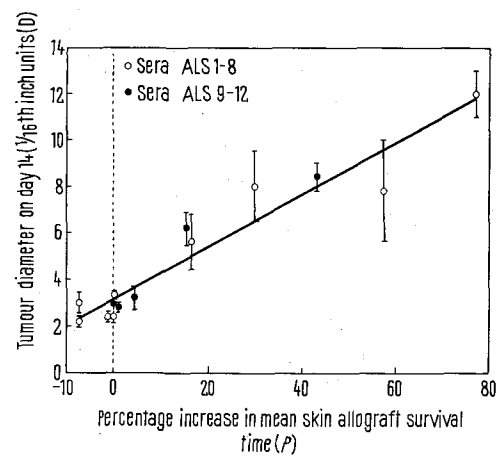

Fig. 3. The relationship between the ability of different batches of ALS to facilitate the growth of tumour allografts and to prolong the survival of skin allografts.

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[23] This investigation was supported by a grant from the Medical Research Council of Great Britain and one of us (ACR) is in receipt of an M.R.C. Junior Research Fellowship.

Prolongation of the duration of survival of skin allografts and facilitation of the growth of tumour allografts in mice treated with various bat ches of normal rabbit serum (NRS) and antilymphocyte serum (ALS)

No.	Serum	Mean survival time of skin allografts in days (MST)	Percentage increase in MST (P)	Tumour diameter on day 14 1/16th inch units (D)	Activity [c]
NRS 1	NRS	14.6 [a]	0.0	3.3 ± 0.2 [d]	—
ALS 1	R33	26.0 [a]	78.0	12.0 ± 1.0	+
ALS 2	IRS 280	14.5 [a]	−0.7	2.4 ± 0.2	—
ALS 3	IRS 49	13.5 [a]	−7.5	3.0 ± 0.5	—
ALS 4	R55	17.0 [a]	16.4	5.6 ± 1.2	+
ALS 5	IRS 28/29	23.0 [a]	57.5	7.8 ± 2.2	+
ALS 6	R53	13.5 [a]	−7.5	2.2 ± 0.2	—
ALS 7	IRS 24	19.0 [a]	30.1	8.0 ± 1.5	+
ALS 8	IRS 282	14.5 [a]	−0.7	2.4 ± 0.2	—
NRS 2	NRS	10.8 [b]	0.0	3.0 ± 0.3	—
ALS 9	ALS 1	12.8 [b]	15.3	6.2 ± 0.7	+
ALS 10	ALG	11.6 [b]	4.5	3.2 ± 0.5	—
ALS 11	NRG	11.2 [b]	0.9	2.8 ± 0.2	—
ALS 12	ALS II	15.9 [b]	43.3	8.4 ± 0.6	+

[a] B. BRADLEY[21]; [b] D. ARKELL[22]; [c] Activity +, active; −, inactive; [d] Standard errors.

Discussion. It has previously been shown that the primary and secondary responses to tumour allografts can be suppressed by treating mice with ALS[14]. Further samples of ALS have now been examined and it has been demonstrated that these vary in their ability to suppress the primary response to tumour allografts. A good relationship has been found between the mean diameter of tumour allografts on the 14th day after implantation and the corresponding percentage mean increase in the survival times of skin allografts. The diameter of tumour allografts on the 14th day is unlikely ever to be very much greater than 12.5 (1/16 inch units) for this model, a limitation being imposed by the maximum growth rate of the tumour. Thus it would not be possible, without dilution, to differentiate sera with:

$$P > \frac{12.5 - 3.21}{0.11} = 85\% \text{ (equation 1).}$$

For all the sera tested $P < 85\%$ although sera which suppress the rejection of skin allografts for periods in excess of 30 days i.e. P between 100% and 250%, have been reported[2,3].

The model which has been investigated serves as a most effective amplifying system for differentiating the immunosuppressive potency of different batches of ALS. For the weakest serum tested the mean survival time of skin allografts was prolonged by 16% whereas the volume of tumour allografts was increased by 388% on the 14th day after implantation.

The measurement of the growth of tumour allografts for screening antilymphocyte sera offers several advantages. Sera can be screened using small groups of animals and only small quantities of serum are required. Active sera can be selected within 2 weeks using extremely simple techniques[23].

Zusammenfassung. Die Wirkung von Kaninchen-anti-Maus-Lymphozytenserum auf das Wachstum von Tumorallotransplantaten kann in kleineren Tiergruppen leicht bestimmt werden, indem man das immunosuppressive Potential verschiedener Serumsätze prüft. Ein gutes Verhältnis zwischen dem durchschnittlichen Durchmesser von Tumortransplantaten am 14. Tag nach dem Implantat und die entsprechende, durchschnittliche, prozentuale Zunahme der Überlebenszeit der Hauttransplantate wird demonstriert.

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The Restoration of the Glycaemic Response to Adrenaline in the Bursectomized Fowl

The importance of the bursa of Fabricius, a lymphoepithelial gland unique to the class Aves, in conferring immunological competence is now well-known[1]. It has also been shown that the bursa influences other systems including modification of the ascorbic acid depletion response of the adrenal glands[2,3], the uptake of iodine by the thyroid glands[4], the activity of xanthine oxidase[5], cytochrome oxidase[5] and the glycogenolytic mechanism of the liver[6], the activity of xanthine oxidase of the kidney[5] and the erythrocyte count and mean corpuscular haemoglobin concentration[7].

Following the demonstration that the glycaemic response of the bursectomized fowl (*Gallus domesticus*) to adrenaline is reduced by one half[6], attempts have been made to restore the responsiveness of the bursectomized bird by treatment with plasma taken from normal intact birds.