of the lymphocytes in the MLC are found in the third fraction. This fraction is composed by the ribosomes and the cell membranes.

These results confirm those of other authors who have found that transplantation antigens are in the ribosomal fraction[9]. The activity of the nuclear fraction is nul in our experiments. It is possible that it is due to the fact that our fraction is purer, which would explain the difference between our results and those of others[10].

The chemical nature of the substance responsible for the transformation of the lymphocytes in the MLC is not determined yet. It is however unlikely that the RNA is involved in this kind of reaction. Further work in this line is in progress[11].

Antigenic activity of the various lymphocyte fractions

3.1	A^a		B^a	2.5
1.6	A^b +	Pellet B 600 *g*	+ B	2
1.9	A^b +	Pellet B 17,000 *g*	+ B^b	2.2
6.5	A^b +	Pellet B 105,000 *g*	+ B^b	2.1
2.7	A^b +	Supernatant	+ B^b	2.5
		A + B^b: 12.5		
		A + B^c: 5.6		

A and B 2 lymphocyte populations; the fractions from B are tested against the living lymphocytes A. [a], 4×10^6 cells/culture; [b], 2×10^6 cells/culture; [c], killed cells. Numbers represent the percentage of transformed cells: mean value of 6 experiments.

Résumé. Les lymphocytes du sang périphérique d'un sujet ont été fractionnés. Trois fractions ont été obtenues. Chaque fraction a été cultivée avec les lymphocytes vivants d'un autre individu non apparenté au premier. Le pourcentage des cellules transformées a été évalué pour chaque culture. Seule la fraction ribosomale dans laquelle se trouvent également les membranes cellulaires est active et donne un pourcentage de transformations comparable à celui obtenu lorsqu'on met en culture dans les mêmes conditions les lymphocytes, entiers et tués par congélation-décongélation, qui ont servi pour la préparation des fractions.

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The Influence of Bacterial Endotoxin on the Formation of Antibody-Forming Spleen Cells in Mice Immunized with Sheep Red Blood Cells

In 1956 Johnson, Gaines and Landy[1] reported that purified lipopolysaccharide preparations from several gram-negative bacterial species are able to enhance antibody production to protein antigens in rabbits. This was repeatedly confirmed by other authors[2]. On the other hand, the same lipopolysaccharide preparations were found to be relatively ineffective in guinea-pigs and mice[1]. Finally, it has been reported that neither bacterial endotoxins nor complete Freund's adjuvants induce adjuvant effects with polysaccharide antigens[3]. That this cannot be considered as a general principle was shown recently[4] for when an accelerated and prolongated multiplication of antibody-forming spleen cells by *Bordetella pertussis* in mice immunized with sheep red blood cells (SRBC) was found. This prompted the authors to explore whether purified bacterial polysaccharides might also be able to increase the number of antibody-forming spleen cells to SRBC in mice under different experimental conditions. For the quantitative determination of plaque-forming spleen cells, the agar technique as described by Jerne et al.[5,6] was employed[4]. As complement guinea-pig serum was used diluted 1:3 (v/v) with physiological saline. For the experiments male albino mice of the inbred strain NMRI/Han. were used.

In a preliminary first investigation 60 mice were divided in 4 groups. The 15 mice of group I were immunized by an i.p. injection of 4×10^8 SRBC while the 15 mice of group II simultaneously with the SRBC received an i.v. injection of 50 μg of a commercially available endotoxin from *Serratia marcescens* (Difco). The immunization schedule of the animals of group III was the same, but each mouse was given an i.v. injection of 100 μg of endotoxin from *S. marcescens*. Finally, the 15 mice of group IV received only an i.v. injection of 100 μg of endotoxin from *S. marcescens*. Three mice out of each group, respectively, were sacrificed at various intervals after immunization and their spleens removed aseptically. The results presented in Table I show that endotoxin effects an accelerated formation of plaque-forming spleen cells. But the number of competent cells was not increased. On the other hand, the sharp decrease of plaque-forming spleen cells between the 7th and 14th day after immunization was delayed in the endotoxin-treated mice. Furthermore, it can be seen in Table I, too, that an endotoxin dose of 100 μg effected only a minute proliferation of hemolysin-forming spleen cells. In order to investigate the adjuvant effect of purified lipopolysaccharide from *S. marcescens* over a longer period of time and by use of greater numbers of mice, a second experiment was undertaken. A collective group of 100 male NMRI-mice was divided into 2 groups. The animals of group I received

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only an i.p. injection of 4×10^8 SRBC, while the 50 mice of group II immunized in the same manner simultaneously were given an i.v. injection of 50 μg endotoxin from *S. marcescens*. Six mice out of both groups, respectively, were sacrificed at various intervals after immunization and the aseptically removed spleens investigated. The results presented in Table II show similarly to those summarized in Table I that the proliferation of plaque-forming spleen cells was accelerated in the endotoxin-treated mice, but no increase in the number of competent spleen cells could be noted. On the other hand, a delayed decrease of plaque-forming cells between the interval from the 7th and 30th day after immunization in endotoxin-treated mice was noted. These findings are not in complete agreement with those from FREEDMAN, NAKANO and BRAUN[7], who reported that hemolysin-forming cells increase following endotoxin injection.

Table I. Effect of endotoxin from *S. marcescens* on the formation of plaque-forming spleen cells in mice immunized by SRBC

Interval after immunization	No. of mice in the groups[a]				Average No. of plaque-forming cells/10^6 spleen cells in the groups			
	I	II	III	IV	I	II	III	IV
2 days	3	3	3	3	49.6	130	139.5	8.2
3 days	3	3	3	3	1659.7	1883.2	1075.4	15.2
7 days	3	3	3	3	640.3	698.4	671.8	3.2
14 days	3	3	3	3	76.2	436.7	325	3.1

[a] Immunization schedule: group I: 4×10^8 SRBC i.p.; group II: 4×10^8 SRBC i.p. + 50 μg endotoxin i.v.; group III: 4×10^8 SRBC i.p. + 100 μg endotoxin i.v.; group IV: 100 μg endotoxin i.v.

Table II. The stimulating effect of endotoxin from *S. marcescens* on the formation of antibody-forming spleen cells in mice immunized by SRBC

Interval after immunization	No. of mice in the groups:[a]		Average No. of plaque-forming cells/10^6 spleen cells in the groups:	
	I	II	I	II
2 days	6	6	52	285
4 days	6	6	1964	1655
7 days	6	6	207	200
14 days	6	6	130	308.8
21 days	6	6	73.1	130.9
30 days	6	6	19.6	34.4
38 days	6	6	23.7	19.9

[a] Immunization schedule: group I: 4×10^8 SRBC i.p.; group II: 4×10^8 SRBC i.p. + 50 μg endotoxin i.v.

Table III. The stimulating effect of endotoxin from *S. marcescens* on low numbers of pre-existing antibody-forming spleen cells

Interval after immunization	No. of mice in the groups:[a]			Average No. of plaque-forming cells/10^6 spleens cells in the groups:		
	I	II	III	I	II	III
1 day	4	4	4	6	5.9	8.5
2 days	6	7	7	14.7	153.6	33.5
4 days	6	7	7	12.4	1803.8	41.8
7 days	4	7	7	14.2	148.8	21.3
10 days	4	4	4	8.4	85.6	27

[a] At first all animals were immunized by an i.p. injection of 4×10^8 SRBC. 31 days later the mice of group II were injected with a second antigen dose of 4×10^8 SRBC while the mice of group III received an i.v. injection of 50 μg endotoxin from *S. marcescens*. The mice of group I were not treated at day 31.

It has been reported, too, that endotoxins can increase pre-existing low levels of specific antibodies. This effect is said to be demonstrated if endotoxin is injected without antigen[8–10]. Thus a third experiment was performed using 100 male NMRI-mice immunized by an i.p. injection of 4×10^8 SRBC. 19 and 31 days after immunization, respectively, 4 mice were sacrificed and from their removed spleens the numbers of hemolysin-producing cells determined using 3 plates per spleen. The average numbers of plaque-forming spleen cells found at the 19th and 31st day after immunization were 46,4 and 14. At the 31st day after the initial immunization, the mice were divided in 3 groups. While the mice of group I received no second antigen injection, the animals of group II were given an i.p. booster injection (4×10^8 SRBC). The mice of group III were i.v. injected with 50 μg endotoxin from *S. marcescens* without antigen. At various intervals after this treatment, mice out of each group were sacrificed and from their removed spleens the numbers of hemolysin-producing spleen cells per million plated spleen cells determined. The results of this experiment – summarized in Table III – show, indeed, that bacterial endotoxin can effect a short-term and moderate proliferation of pre-existing low numbers of antibody-forming spleen cells. Further experiments indicate such effects can be as well produced by i.p. injection of pertussis organisms as by i.p. injected incomplete and complete Freund's adjuvant[11].

Zusammenfassung. Es wird berichtet, dass die simultane i.v. Injektion von *Serratia marcescens*-Endotoxin mit i.p. verabfolgten Schafbluterythrocyten bei weissen Mäusen zu einer beschleunigten Bildung hämolysinbildender Milzzellen und zu einem verzögertem Abfall dieser kompetenten Zellen zwischen dem 7. und 30. Tage nach der Immunisierung führt. Eine echte Vermehrung plaquebildender Milzzellen wurde jedoch nicht gefunden. Weiterhin liess sich zeigen, dass die alleinige i.v. Injektion von bakteriellem Lipopolysaccharid eine mässige Proliferation von in geringer Zahl vorhandenen antikörperbildenden Milzzellen bewirkt.

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