Positive Immunological Reaction of Gut Cells from Orally Immunized Animals Demonstrated by Passive Cutaneous Anaphylaxis

The oral immunization of animals as described in various papers by RAETTIG[1,2] and resumed in a series of 5 papers[3–7], always lacked the demonstration of adequate antibody level or immunologically competent cells. In spite of a great protection index in mice[3,5,7] and during an epidemic[1], achieved by orally introduced antigens, it was only possible by the hemagglutination technique to demonstrate the presence of low antibody titers.

ROSENBERG, CHANDLER et al.[8] demonstrated the possibility of showing immunologically active cells when these were implanted intracutaneously into normal recipients, and the possibility of detecting the presence of antibody by inducing the passive cutaneous anaphylaxis (PCA) reaction with excess of antigen and dye as indicator. This communication presents results obtained with gut cells, from orally immunized guinea-pigs, implanted in recipients, guinea-pigs and rats, and challenged with the antigen used for immunization.

Twenty guinea-pigs of Pirbright white W.58 strain were orally immunized with a heat inactivated (100 °C for 5 min) germ suspension[7] containing a mixture in equal parts of *Salmonella typhi murium* strains 6616, 6639, 6643, 6656, 6659 RKTCC, total germ cell count was 10^9 ml. The animals were fed with 1 ml of this suspension every day for 10 days.

Sixteen days after the last immunization dose the gut and spleen were removed and prepared for the PCA technique. Gut and spleen pieces were gently homogenized with a micro tissue grinder in a chilled Eagle medium. The suspension was filtered through a cheesecloth and the nucleated cells counted in a hemocytometer chamber by adding 10% of a Giemsas 1% solution in 1 *M* citric acid. Adequate quantity of cell suspension were obtained by dilution or gentle centrifugation at 4 °C for 5 min at 1500 rpm and resuspension in an adequate volume.

The animals used as recipients were normal Pirbright white W.58 strain guinea-pigs weighing 250 ± 50 g and normal white rats weighing 200 ± 50 g. Each animal was sensibilized i.c. into the flank at 3 or 4 points. Volumes of 0.1–0.3 ml were injected with a needle 20 (0.45 × 21 mm) short bevel. A maximum of 30 min elapsed between excision of the donor tissues and injection of the cell suspension into the recipients. The animals were challenged in triplicates at the following times: 2, 24, 48, 96, 120 h. The antigen for challenge was prepared by freezing and thawing the germ cell suspension (10^9 cells ml) until no more living cells were present as proved by the sterility test. The challenge i.v. injection contained 0.5 ml of this antigen and 0.5 ml of a 0.5% Evans blue solution in saline. The guinea-pigs were challenged through the ear vein and the rats through the tail vein with a 0.30 × 16 mm needle. 20 min after challenge recipients were sacrificed. The skin was inverted and sites were examined for the dye location, the lesions diameter was measured with a transparent ruler. Gut and spleen cells from non-immunized guinea-pigs were used as control.

As can be seen from the Table, the implanted gut cells are able to evidence a positive PCA reaction after more than 24 h, with a maximum reaction between 72 and 96 h. The reaction when challenged 2 h after sensibilization is negative in all instances. The intensity of the reaction is

1 H. RAETTIG, Z. ImmunForsch. exp. Ther. *108*, 165 (1950).
2 H. RAETTIG, Zentbl. Bakt. ParasitKde *183*, 1 (1962).
3 H. RAETTIG, Zentbl. Bakt. ParasitKde *193*, 398 (1964).
4 H. RAETTIG, Zentbl. Bakt. ParasitKde *197*, 368 (1965).
5 H. RAETTIG, Zentbl. Bakt. ParasitKde *203*, 478 (1967).
6 H. RAETTIG and A. BUSE, Zentbl. Bakt. ParasitKde *204*, 221 (1967).
7 B. LIETZ and H. RAETTIG, Zentbl. Bakt. ParasitKde *204*, 543 (1967).
8 L. T. ROSENBERG, M. H. CHANDLER and E. E. FISCHEL, J. Immun. *81*, 136 (1958).

Immunological reaction of cells from orally immunized animals

Donor of cells			Recipient				Interval between transfer and challenge, h					
Species of animal cell donor	Antigen orally introduced	Interval between last oral immunization and gut removal Days	Species of animal cell receptor	No. of animals	No. of cells injected	Total sides sensibilized	2	24	48	72	96	120
GP	STM	16	GP	4	1.5×10^6	12	O	++	+++	++++	++	O
GP	STM	16	GP	4	1×10^6	12	O	+	+++	++++	±	O
GP	STM	16	GP	4	1.5×10^5	12	O	+	++	+++	+	O
GP	STM	16	GP	4	1×10^5	12	O	±	++	+++	+	O
GP	STM	16	GP	4	1.5×10^4	12	O	O	+	+	O	O
GP	STM	16	GP	4	1×10^4	12	O	O	±	O	O	O
GP	STM	16	R	4	1×10^8	12	O	±	+	++	+	O
GP	STM	16	R	4	1×10^7	12	O	±	+	+	O	O
GP	STM	16	R	4	1.5×10^6	12	O	O	O	O	O	O
GP	STM	16	R	4	1×10^6	12	O	O	O	O	O	O
GP	STM	16	R	4	5×10^5	12	O	O	O	O	O	O
GP	STM	16	R	4	1×10^5	12	O	O	O	O	O	O

GP, guinea-pig; R, rat; STM, heat inactivated *Salmonella typhi murium*; O, no reaction; +, less than 5 mm ϕ; ++, 5–10 mm ϕ, +++, 10–15 mm ϕ.

a function of the quantity of cells implanted. The implantation of guinea-pig cells to rats is not so effective as in the homologous animal. To obtain a blue lesion of 5–10 mm in rats implanted with orally immunized guinea-pigs gut cells, we need 10^8 nucleated cells whereas the same effect is obtained in homologous animal with approximately 10^6 cells. We did not succeed in obtaining a positive PCA with spleen cells from immunized animals, nor from gut or spleen cells from normal non-immunized guinea-pigs. In all our registered experiments, the PCA reaction was negative after 120 h.

Experiments to obtain a positive PCA when challenging with the following antigens: *Escherichia coli* 2380 RKTCC, *Sh. flexneri* 2a RKTCC and bovine serum albumin, were unsuccessful in all instances.

In a total of 20 animals immunized, 6 did not show positive reaction in gut nor in the spleen tissues.

Oral ingestion of antigens leading to stimulation of some immunological reaction is generally accepted with doubt. Therefore we looked for a technique that can evidence some reaction between the antigen and the orally immunized animal.

Gut cells of immunized animals showed positive PCA reactions when challenged with the homologous antigen and negative reaction when other antigens were used. This demonstrates that this reaction was highly specific. The specificity is probable also as related to the species of the animal, as in rats comparatively high cell quantities must be used to obtain positive results. It is also interesting that 30% of the animals could not show a positive PCA reaction in the gut cells. This may be due to a phenotype individual ability to be stimulated by oral immunization. Further work to elucidate the nature of this reaction after oral immunization is in progress.

Zusammenfassung. Meerschweinchen wurden mit hitzeinaktivierten *S. typhi murium*-Bakterien oral mit Sonde immunisiert. 16 Tage nach der Impfung wird diesen Tieren der Dünndarm entnommen, zerkleinert und im passiven, kutanen Anaphylaxie-Test (PCA) in unbehandelten Meerschweinchen und Ratten auf Antikörperbildung geprüft. Im Dünndarm von zwei Dritteln der oral geimpften Meerschweinchen wurden hochspezifische Reagine mit der PCA-Technik nachgewiesen.

Y. Levanon[9], H. Raettig and S. M. O. Rossetini[9]

Robert-Koch-Institut im Bundesgesundheitsamt, Abt. f. Bakteriologie und Seuchenforschung, Berlin (Germany), 30 January 1968.

[9] Invited for research plan by the Senator für Gesundheitswesen, Berlin. Permanent address: Instituto Adolfo Lutz, S. Paulo, Brazil.

Production d'anticorps 7 S et 19 S chez des Rats d'âge différent

Dans un travail publié antérieurement[1,2] nous avons montré qu'après injection d'antigène protéique à 2 échantillons de rats Wistar d'âge différent, la production d'anticorps des animaux âgés était inférieure à celle des animaux jeunes.

Dans le présent travail, nous avons cherché à confirmer et à préciser nos premiers résultats en variant les échantillons et le mode d'immunisation, et en dosant les anticorps avant et après action du 2-mercaptoéthanol. On a montré en effet que les anticorps de poids moléculaire élevé (19 S) qui caractérisent la première phase de la réponse primaire sont sensibles à l'action du 2-mercaptoéthanol et que les anticorps de poids moléculaire plus faible (7 S) qui caractérisent la seconde phase, résistent à cet agent réducteur[3–7].

Conditions expérimentales. Deux expériences furent entreprises indépendamment, la première portant sur la synthèse des anticorps 7 S et 19 S au début de l'immunisation (40 premiers jours), la seconde sur l'évolution des taux d'anticorps au cours d'une période plus prolongée (du 10e au 120e jour) et après injection de rappel (60e jour).

Dans ces 2 expériences, nous avons immunisé des rats Wistar (WCF) femelles, avec de la sérum albumine bovine (SAB) (Behring-Werke). Chaque animal recevait 1 mg de SAB émulsionné dans de l'Adjuvant de Freund complet (Difco); volume final: 1 ml. L'injection était effectuée dans le coussinet plantaire: 0,15 ml dans chaque patte antérieure, 0,35 ml dans chaque patte postérieure. Des prises de sang ont été pratiquées avant l'immunisation et à des temps variés après injection de l'antigène. Tous les sérums – conservés à l'état congelé – ont été étudiés avant et après traitement par le 2-mercaptoéthanol (2-ME), à la concentration finale de 0,1 *M*, pendant 1 h à 37 °C.

La teneur en anticorps a été déterminée par hémagglutination passive[8,9]. Chaque titrage a été effectué en 4 exemplaires. La fraction sensible au 2-ME a été appelée «19 S» et la fraction résistante «7 S». Afin de réaliser les meilleures conditions de comparaison, nous avons toujours titré simultanément dans les 2 expériences une série de sérums d'animaux jeunes et une série de sérums d'animaux âgés correspondant à tous les temps étudiés.

Résultats. (A) Synthèse des anticorps 7 S et 19 S au début de l'immunisation.

Pour cette expérience, nous disposions d'un échantillon de 24 animaux jeunes (10 semaines) d'un poids moyen de 160 g et d'un échantillon de 24 animaux âgés de 16 mois, d'un poids moyen de 325 g. Chaque échantillon a été divisé en 3 lots de 8 animaux chacun, saignés respectivement les 3e et 20e jours, les 6e et 30e jours, les 10e et 40e jours après l'injection de l'antigène.

[1] Ph. Goullet et H. Kaufmann, Experientia *21*, 46 (1965).
[2] Ph. Goullet et H. Kaufmann, Gerontologia *10*, 76 (1965).
[3] H. F. Deutsch et J. I. Morton, Science *125*, 600 (1957).
[4] J. W. Uhr et M. S. Finkelstein, J. exp. Med. *117*, 457 (1963).
[5] Y. B. Kim, S. G. Bradley et D. W. Watson, J. Immun. *93*, 798 (1964).
[6] J. W. Uhr, Science *145*, 457 (1964).
[7] G. W. Santos et A. H. Owens jr., Nature *209*, 622 (1966).
[8] S. V. Boyden, J. exp. Med. *93*, 107 (1951).
[9] A. B. Stavitsky, J. Immun. *72*, 360 (1954).