IMMUNOGENIC AND SENSITIZING PROPERTIES OF SEXUAL RECOMBINANTS OF Escherichia coli AND Shigella flexneri

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Sexual recombinants of organisms of the Shigella genus and of Escherichia coli group Hfr H have been known to experimental workers for more than 10 years. Some investigators have isolated directly from patients strains close in their basic bacteriological characteristics and antigenic structure to these sexual hybrids.

Nevertheless the role of genetic recombinants in the pathogenesis of bacterial dysentery is not clear. However, from the viewpoint of their immunogenic activity the genetic hybrids of Shigella and Escherichia are interesting in connection with the possible intensification of this activity as a result of the supplementing of the antigenic basis of the recipient strains with donor's genetic material.

The object of the present investigation was to compare the immunogenic and sensitizing properties of sexual recombinants and the initial strains of Escherichia and Shigella.

EXPERIMENTAL METHOD

The test material consisted of four strains of Shigella flexneri (Nos. 737, 751, 2047, and 2155), actively in sexual contact with E. coli cells and yielding a progeny of genetic recombinants, E. coli strain Hfr H, used in the sexual crossing experiments, and 16 strains of genetic hybrids obtained by the classical method on a minimal synthetic medium with lactose, streptomycin, and an eosin-methylene indicator.

The immunogenic properties were investigated in the experiments of series I by preliminary immunization of albino mice with heat-killed dysentery recombinant vaccines and preparations of E. coli Hfr H. The whole group of experimental animals (1350 individuals) was subdivided into nine groups with five subgroups. Each subgroup consisted of 30 animals. Immunization was by two intraperitoneal injections at an interval of 1 week, in equal doses: 1.5 billion bacterial cells for the primary injection and 2 billion bacterial cells for the reimmunization. Ten days after immunization, the experimental animals were inoculated with 1 and 2 LD_{50} of living virulent cultures suspended in semiliquid minimal agar.

EXPERIMENTAL RESULTS

The experimental results are given in the table.

Immunization with donor strains E. coli Hfr H gave protection only against the homologous strain of Escherichia. Protection against infection by recombinant strains and by homologous and heterologous strains of S. flexneri and Shigella sonnei was practically absent.

The dysentery strains used for preliminary vaccination of the animals created a fairly stable immunity to homologous and heterologous strains of S. flexneri. The survival rate after infection of the animals varied from 87.7 to 96.7% for homologous strains and from 50 to 90% for heterologous strains of this species. The immunity was strictly species-specific and type-specific in character and did not extend to S. sonnei. The degree of protection of the animals of this group against genetic-recombinant bacteria likewise was fairly high, but lower than the degree of protection of the animals against homologous Shigella strains, and was close to the degree of protection given against heterologous strains of S. flexneri.

The recombinant strains, from the immunogenic aspect, merely repeated the recipient strains of dysentery bacteria, despite their common antigenic specificity with the parent strains – Escherichia and Shigella. Vaccination with them led to a survival rate of 89.4-96.6% among the animals when they were subsequently infected with

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Immunogenic Properties of Sexual Recombinants Compared with Initial Strains after Inoculation of Animals with 2 LD_{50} of Living Cultures

Strains used for immunization (heat-killed vaccines)	Survival rate of mice (in %) after innoculation with				
	homologous recombinant strain	donor strain E. coli Hfr H	recipient homologous strain	heterologous strains of S. flexneri*	Shigella sonnei
E. Coli Hfr H	—	86,7	—	—	—
Shigella flexneri 737	90,3	—	96,7	90,0	—
Recombinants 737	96,6	—	95,7	87,5	—
Shigella flexneri 2047	84,2	—	87,7	50,0	—
Recombinants 2047	92,7	—	91,5	93,3	—
Shigella flexneri 2155	87,4	—	96,7	91,0	—
Recombinants 2155	91,8	—	90,2	90,0	—
Shigella flexneri 751	77,5	—	90,0	80,0	—
Recombinants 751	89,4	—	73,0	70,2	—

*S. flexneri No. 15168 with high virulence.

24-hour cultures of the same hybrid strains. Protection of the animals against the corresponding recipient dysentery bacteria also was close to this figure, varying within the range 73.0-95.8%.

Infection of animals immunized with genetic recombinants by the donor strain E. coli Hfr H was lethal in every case. The impression actually was obtained that immunization with the recombinants hastened the lethal outcome. In other words, despite the appearance of new substances and structures in the sexual recombinants, and especially of the enzyme β-galactosidase, which possesses antigenic properties of its own, their immunogenicity was not increased but merely approached in intensity the immunogenicity of the original recipient strains of S. flexneri.

Parallel with the immunization of the animals, in identical conditions the virulence of the strains used for preparing the vaccines and for subsequent infection of the animals was determined. During successive trials of the LD_{50}, some of the albino mice survived. The animals remaining alive after use in determining the virulence of the cultures were regarded after 1 month as having been immunized with living vaccines. Cross experiments were carried out on these animals and the immunogenic properties of the living cultures were verified. As in the experiments of series I, the experimental albino mice were infected with 1 and 2 LD_{50} of homologous recombinant donor and recipient strains and heterologous strains of Shigella. The following results were obtained, with no particular dependence on the primary dose of living bacteria.

The animals which survived dysentery infection following primary inoculation with recipient strains of Shigella, remained healthy after reinoculation with homologous and heterologous strains and recombinant strains. They died after 72-96 h only if infected with E. coli Hfr H. Albino mice which survived after primary inoculation with E. coli Hfr H or recombinant strains were completely unprotected against dysentery infection, even if produced by inoculation with 0.5 and 0.2 LD_{50}. These doses had no effect on normal mice. The background created by E. coli Hfr H was especially unfavorable. As a rule the animals died at the end of the 1st or, more rarely, in the middle of the 2nd day after infection. Possibly this phenomenon could be associated with paraspecific allergization. Nothing like it was observed following analogous intraperitoneal immunization with heat-killed cultures. Heating appeared to destroy the allergizing effect produced by the living, proliferating bacteria.

The sensitizing action of both E. coli Hfr H and of the recombinant strains suggested a genetic transmission of the allergizing principle.

To verify and confirm these observations a further study was made of the sensitizing properties of the donor strain E. coli Hfr H and of its genetic recombinants with the use of allergic tests.

In the experiments of series I rabbits were used, as they are considered by some authorities [1] to be the most sensitive biological model, and 15 of these animals were immunized by repeated subcutaneous injections of small doses of the same heat-killed vaccines. After 2 or 3 injections a zone of infiltration developed at the site of injection, and the tissue became erythematous, and later, necrotic – the result of summation of the specific toxic-sensitizing action as in the Arthüs phenomenon.

In the experiments of series II (14 rabbits) the preliminary subcutaneous immunization with heat-killed vaccines from the donor strain E. coli Hfr H or from genetic recombinants of S. flexneri, followed by injection of heat-

killed dysentery vaccine foreign for the first and relatively foreign for the recombinant strains, also was accompanied by the appearance of swelling, erythema, and a zone of necrosis, although it was somewhat less marked (the Schwartzmann-Sanarelli phenomenon).

The results obtained show evidence of a specific adaptation of the experimental animals in the process of vaccination: besides immunological changes, manifestations are seen of the allergizing action of the vaccine antigens, with the character both of specific sensitization and of para-allergy. Consequently, the allergization of the experimental animals takes place under the influence not only of living, but also of heat-killed cultures. This was observed in rabbits, in contrast to mice, in the form of purely local, rather than general, manifestations. Consequently, the original hypothesis suggesting a thermolabile allergic component was not quite correct. Heating the culture probably only depressed its sensitizing properties. In this case it was a matter not so much of a change in the properties of the allergen during preparation of the heat-killed vaccine as of the stabilization of its amount.

Other interesting observations were made in experiments in which the keratoconjunctival test was carried out on guinea pigs (experiments of series III).

When applied to the conjunctiva of a guinea pig, cells of E. coli Hfr H, even in a very high concentration, did not give rise to the phenomena of keratitis and keratoconjunctivitis. Only in 2 of 13 cases was a mild catarrhal inflammation of the conjunctiva seen. Performance of the keratoconjunctival test with S. flexneri on the same animals 1 month after their infection with E. coli was accompanied by the rapid development of keratoconjunctivitis with a prolonged and persistent course. In these circumstances a positive keratoconjunctival test was even obtained with S. flexneri after storage in the laboratory (on nutrient media) for 6 and 9 months. Three months before the present experiments these strains no longer gave the typical keratoconjunctival reaction in guinea pigs, although when tested immediately after isolation from the patient a positive reaction was obtained.

A similar sensitizing action, although weaker, was given by the recombinant strains. Not one of these sexual hybrids gave an independent keratoconjunctival reaction, but they had a preparatory, sensitizing action, like the donor strain E. coli Hfr H. However, in 3 of 16 cases the old genetic hybrids, after storage for 3 months, caused an acute keratoconjunctival reaction, but only after sensitization of the conjunctiva.

The formation of para-allergy and of specific allergy in the course of successive infection with E. coli and Shigella strains, and also with recombinant and Shigella strains (experiments of series IV), was confirmed by allergic tests both on guinea pigs surviving infection and on animals surviving after tests of immunogenicity.

The allergens used were a dysentery allergen from Shigella strains (manufactured by the Leningrad Institute of Vaccines and Sera), an E. coli allergen, and recombinant allergens prepared by the author from E. coli Hfr H and hybrid strains by the acid hydrolysis method [2].

The results of intradermal allergic tests showed that, regardless of the biological model used, a previous infection with E. coli or with recombinant facilitated the development of a state of specific allergy and para-allergy in the animal.

Hence, E. coli Hfr H and the recombinant strains formed by sexual contact between E. coli Hfr H and S. flexneri constitute an important pathogenetic factor which supplements the allergic link in the development of dysentery infection in laboratory animals.

It is possible that relationships such as this between microorganisms of different species and also of intermediate forms, and their combined influence on the reactivity of the host may have an important action on the character of the course of bacterial dysentery and may be the cause of the development of atypical forms of dysentery and of frequent relapses of chronic dysentery.

The positive result of the diagnostic allergic test with dysenterin, because of these observations, cannot be regarded as the result of the action of true Shigella cells alone on the host. The allergic test may also give an indication of cross-sensitization with genetically related microorganisms, including the sexual hybrids of the dysentery bacteria.

SUMMARY

The genetic sexual recombinants of S. flexneri and E. coli in the process of immunization of animals confer immunity only to the recombinant forms, in a lesser degree to the recipient homo- and heterologous strains of Shigella, and fail to afford protection against E. coli Hfr H and other types of dysentery bacteria. A donor of E. coli Hfr H contributes to the formation of a strictly specific immunity failing to protect animals either against sexual hybrids

or against Shigella. A living culture of E. coli Hfr H and genetic recombinants possesses marked allergie qualities with regard to various biological models: rabbits, albino mice, guinea pigs.

Sensitization is devoid of any strict specificity and has a cross para-allergic character. Genetic hybrids and E. coli donors are an important pathogenetic factor supplementing the allergic link in the development of bacterial dysentery.

LITERATURE CITED

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