numbers of thymocytes rendered anti-SV serum specific only for the rat bone marrow B lymphocytes.

In immunofluorescence assays, thymocytes exhibited brilliant green fluorescence of the ring-type (figure 2, a). The brain sections treated with anti-SV[5] showed an accumulation of specific fluorescence in the nerve-cell body, whereas the network of fibres exhibited a less intense fluorescence (figure 2, b). The neuronal nuclei, pia, choroid plexus, cilia of the ependymal lining and brain blood vessels remained virtually unstained. B lymphocytes exposed to anti-SV serum absorbed with 10^9–10^{10} thymocytes displayed a discontinuous specific staining of the cell membrane (figure 2, c and d). Anti-SV thus absorbed stained also cells of the plasmacytic series, and this fluorescence was characterized by a concentration of fluorescein-conjugate in the region of the excentric nucleus (figure 2, e and f).

Discussion. The most important finding made in this study concerns the common antigenic markers of rat brain cells (i.e. synaptic vesicles) and B lymphocytes. Thus, anti-SV absorbed with 10^9–10^{10} thymocytes reacted only with rat B lymphocytes in cytotoxicity and immunofluorescence tests, although being completely inactive for thymocytes. It should be argued that this B-specific anti-SV serum can also act on lymphohaemopoietic stem cells of the rat bone marrow. This possibility was excluded by absorbing anti-SV serum with the rat fetal liver[9]. These results also suggest that the rat bone marrow contains a 'null' subpopulation of B lymphocytes[11] which cannot be detected with properly absorbed rabbit anti-SV serum[14].

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Passive transfer of resistance in rabbits infested with adult *Ixodes ricinus* L.: Humoral factors influence feeding and egg laying[1]

M. Brossard and P. Girardin

Institut de Zoologie, Université de Neuchâtel, CH-2000 Neuchâtel (Switzerland), 19 January 1979

Summary. Partial immunity against the bites of ♀♀ *I. ricinus* was transferred to normal rabbits by inoculating immune serum from resistant animals. Transferred humoral factors diminished the weight of the ticks' blood meal by 29% and increased the feeding period by about 1 day in comparison with ectoparasites engorged on controls. They provoked also the failure of egg laying by ♀♀ *I. ricinus*. Only 55% of ticks fed on treated rabbits laid eggs (94% on controls). The immunological state of immune serum donors or recipients was studied and the IgG and homocytotropic specific anti-*I. ricinus* antibodies were identified. The immediate hypersensitivity of rabbits' skin was also controlled.

In a previous paper, it was found that rabbits acquired progressively a resistance against the bites of ♀♀ *I. ricinus* as a result of repeated infestations by this tick[2]. This 'immunity' was reflected by increased mean feeding time, inadequate blood meals and poor oviposition. Thus, after 4 tick infestations, only 25% of all ticks fed normally, and as high as 84% of females failed to lay eggs. It was also shown that transfer of immune sera to normal rabbits affected the feeding of ticks; females ingested 25% less blood than did ticks fed on control rabbits[3].

In the present study, an attempt was made to increase the transferred resistance by inoculating larger volumes of immune serum. This treatment not only influenced the amount of ingested blood but also the feeding time and the production and laying of eggs. Efforts were made to identify and evaluate the anti-*I. ricinus* antibodies (IgG class or homocytotropic), and, finally, to determine the rabbits' skin sensitivity against allergens prepared from ♀♀ *I. ricinus*.

Table 1. Effects of humoral factors on feeding of ♀♀ *I. ricinus*

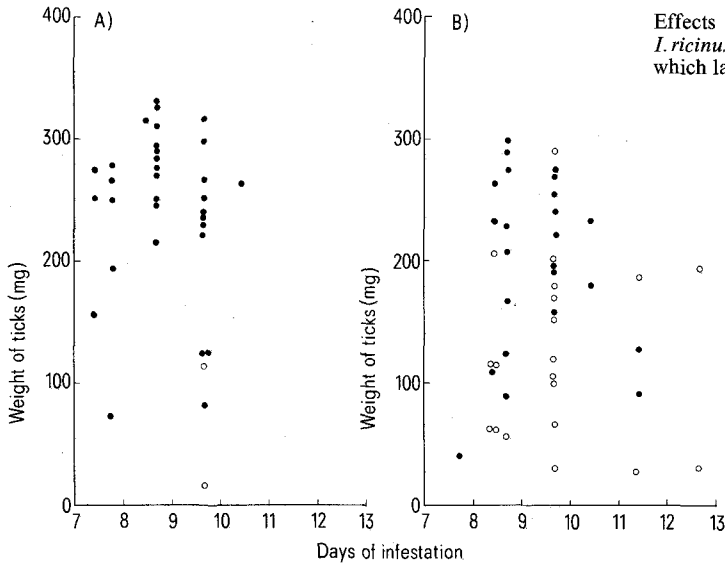
	On control rabbits n = 34	On treated rabbits n = 44
Mean weight of fed ♀♀ (mg)	231 ± 73	165 ± 81
Mean feading time (h)	173 ± 20	190 ± 27

n = Number of fed ♀♀ *I. ricinus*.

Table 2. Immunological state of donors and recipients of immune serum

	Control rabbits number				Treated rabbits number				
	1	2	3	4	5	6	7	8	9
IgG titre (day 4)	0	0	0	0	1/80	1/80	1/80	1/80	1/80
IgG titre (infestation end)	1/40	1/20	1/20	1/20	1/160	1/80	1/80	1/80	1/160
Homocytotropic antibodies titre (day 4)	0	0	0	0	1/1	1/3	1/1	1/1	1/3
Homocytotropic antibodies titre (infestation end)	0	0	0	0	1/3	1/9	1/3	1/3	1/9
Skin's sensibility (infestation end)	−	−	−	−	+	+	+	+	+

Transferred immune serum: IgG titre = 1/640; homocytotropic antibodies titre = 1/81; + = positive cutaneous test; − = negative cutaneous test.



Effects of humoral factors on feeding and egg laying of ♀♀ *I. ricinus*. *A* Control rabbits, *B* treated animals. ● Fed females, which laid eggs. ○ Fed females, which did not lay eggs.

Materials and methods. 5 male rabbits of a Russian race (Himalayan breed, genotype aac^Hc^H), weighing approximately 2 kg, were used. They were injected twice with 15 ml of immune serum, 24 h apart, in the marginal vein of the ear. The titre of anti-*I. ricinus* circulating antibodies of injected immune serum (IgG class) was determined by indirect immunofluorescence[2,4]. It was high, i.e. 1/640. The titre (1/81) of homocytotropic specific antibodies was measured by a Prausnitz-Küstner test[5], by injecting the allergen 72 h after the skin's sensibilization. The antigen utilized consisted of an aqueous extract of ♀♀ *I. ricinus* salivary glands, dissected 5 days after the beginning of the blood meal[6]. Each dose of allergen injected intradermally contained 10^6 pg of proteins, as determined by the technic of Kalckar[7]. To visualize the increased permeability of the venules in the skin, Evans Blue (2%) was injected i.v. 30 min before challenge.

4 h after the first injection of immune serum, each rabbit was infested with 10 females and 10 males *I. ricinus*. These ticks originated from colonies maintained in our institute[8]. As controls, 4 rabbits, injected twice with serum from rabbits without previous ticks' infestation, were used. These animals were then infested with ticks as were the treated animals.

The titres of IgG and homocytotropic specific antibodies of the recipients were measured as described before on day 4 and at the end of tick infestation, i.e. between the 13th and 15th day after the placement of ticks. The skin sensitivity (Prausnitz-Küstner immediate type) of the recipients was determined by a direct cutaneous test. The allergen utilized was the same as that prepared for the Prausnitz-Küstner test.

The effects of treament on biology of ♀♀ *I. ricinus* have been statistically analysed. With a χ^2 test for contingency tables, we have compared the control and treated rabbits for: a) the distributions of engorged ticks' weight. Intervals class were 60 mg, except for the last (120 mg); b) the distributions of ticks' feeding time. Intervals class were 1 day, except for the last (3 days); c) the proportions of ticks laying eggs. The contingency tables can be reconstituted from the figure.

Results. The injections of immune serum had adverse effects on the ♀♀ *I. ricinus* feeding and egg laying (figure). Thus, the weight distribution of ticks engorged on control or treated rabbits differed ($\chi^2 = 14.40$, ndf 3). The feeding time distribution of ticks was also modified by the treatment ($\chi^2 = 11.46$, ndf 3). Fed on control rabbits, nearly all the ♀♀ *I. ricinus* laid eggs (32/34 or 94%). Fed on treated rabbits, this proportion was dramatically diminished (24/44 or 55%). The observed difference was highly significant ($\chi^2 = 20.42$, ndf 1).

Differences were also observed between the means of ticks' weight and between the means of ticks' feeding time (table 1). Thus, ♀♀ *I. ricinus* fed on treated rabbits weighed only 165 ± 81 mg, i.e. 66 mg less than the ticks fed on control animals (231 ± 73 mg). On treated animals, the mean feeding time was lengthened by about 1 day (173 ± 20 h on controls, 190 ± 27 h on treated animals).

The 2 injections of immune serum provoked an early appearance of IgG and homocytotropic antibodies. 3 days after the beginning of tick infestation, the titres of IgG antibodies were 1/80 in all cases and the titres of homocytotropic antibodies were between 1/1 and 1/3 (table 2). At the end of the infestation, these titres were between 1/80 and 1/160 and between 1/3 and 1/9 respectively. In control rabbits, IgG antibodies were not detectable until the end of the tick feeding. The titres then ranged between 1/20 and 1/40. In all these animals, no homocytotropic antibodies were demonstrated during and after the infestation.

By transferring immune serum, we were also able to sensitize the rabbits' skin against the allergens in the saliva of ♀♀ *I. ricinus*. Indeed the treated rabbits developed an immediate local hypersensitivity after intradermal injection of antigen (table 2). On the control animals, all cutaneous tests were negative.

Discussion and conclusion. Inoculation of immune serum, in quantities larger than previously reported[3], resulted in far better immune reactions. This was indicated by the facts that *I. ricinus* placed for feeding on treated rabbits ingested 29% less blood during a feeding period that was generally about 1 day longer than that of ticks engorged on control rabbits. Similarly, only 55% of such ticks laid eggs, whereas 94% of females fed on control rabbits oviposited.

The nature of this 'immunity' acquired by transfer is not as yet fully understood. We may only speculate that humoral factors play a role. A partial resistance caused by transfer of immune plasma was also recorded for cattle infested with *Boophilus microplus*[9]. On the other hand, in guinea-pigs infested with *Dermacentor andersoni* larvae, only lymph node cells from resistant animals conferred good immunity[10]. Therefore, treatment with cyclophosphamide decreased acquired resistance of guinea-pigs, a phenome-

non suggesting that humoral factors could indeed take part in the development of this immunity[11].

At the 4th day of our experiment, all recipients of immune serum developed rather good IgG titres (1/80, table 2) against *I. ricinus*, indicating perhaps involvement of circulating antibodies of that immunoglobulin class. Circulating homocytotropic specific antibodies seemed to be more diluted. In fact the titres were only 1/1 or 1/3 in contrast to 1/81 in the transferred immune serum. These differences in quantities of circulating antibodies may be due to the different physiological destination of IgG and homocytotropic antibodies, the latter fixing normally to receptors of basophils or tissue mast cells. Indeed, the skin of all treated rabbits proved to be sensitized to *I. ricinus* allergens (table 2). Studies are under way to determine the role which this immediate hypersensitivity may play in the host's acquired resistance to *I. ricinus*.

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Structure specificity of some immunoadjuvant synthetic glycopeptides

K. Mašek, M. Zaoral, J. Ježek and V. Krchňák

Institute of Pharmacology, Czechoslovak Academy of Sciences, Albertov 4, 12000 Praha 2, and Institute of Organic Chemistry and Biochemistry, Czechoslovak Academy of Sciences, Praha (Czechoslovakia), 21 December 1978

Summary. The immunoadjuvant activity of muramyl dipeptide seems to be critically dependent on the type of substitution of the γ-carboxyl group of the D-isoglutamine residue. Moreover the nonapeptide L-Ala-D-isoGlu-L-Lys-D-Ala-$(Gly)_5$-OME also shows a definite effect.

The identification of N-acetyl muramyl L-alanyl-D-isoglutamine as the minimal structure required for immunoadjuvant activity[1] opened the way for a systematic study of structure-activity relationships. Previous studies have established that the adjuvant activity is critically dependent on the presence of the D-configuration of glutamic acid residues, and on the amidation of α-carboxyl group of this amino acid[2-5]. The main objective of the present work was to extend the above mentioned original findings.

Material and methods. Compounds listed in the table were prepared as described elsewhere[6,7]: The peptides both by liquid and solid-phase peptide synthesis, the glycopeptides and glycoamino acid by acylation of the corresponding peptides or aminoacid derivative with 1-α-0-benzyl-4,6-0-benzylidenemuramic acid. The cleavage of the protecting groups was effected by hydrogen bromide in acetic acid[8] (peptides) or by sodium in liquid ammonia (glycopeptides)[6]. The immunoadjuvant activity was assayed on female albino guinea-pigs injected in the left hind footpad with 0.2 ml of water-in-oil emulsion (Drakeol, Arlacel and saline 4:1:5) containing 2.5 mg of crystalline ovalbumin and 200 μg of synthetic peptides or glycopeptides, or 1 mg of

Induction of delayed hypersensitivity to ovalbumin in guinea-pigs by synthetic peptides and glycopeptides

Compound tested	Dose (μg)	Skin response (24 h)
Freund's incomplete adjuvant (FIA)	-	7.5 ± 0.8
Freund's complete adjuvant (FCA)	1000	13.6 ± 0.9*
FIA + L-Ala-D-Glu-NH_2 ┌ Lys(Ac)-D-Ala-NH_2	200	7.6 ± 1.5
FIA + L-Ala-D-Glu-NH_2 ┌ Lys(Ac)-D-Ala-$(Gly)_5$-OME	200	7.3 ± 1.0
FIA + Ala-D-Glu-NH_2	200	11.5 ± 1.1*
FIA + NAM-Ala-NH_2	200	5.3 ± 0.5
FIA + NAM-Ala-D-Glu-NH_2	200	12.7 ± 1.5*
Freund's incomplete adjuvant	-	4.5 ± 0.4
Freund's complete adjuvant	1000	12.6 ± 1.5*
FIA + NAM-Ala-D-Glu-NH_2 | NH_2	200	11.2 ± 0.5*
FIA + NAM-Ala-D-Glu-NH_2 ┌ Lys(Ac)-D-Ala-NH_2	200	11.4 ± 1.2*
FIA + NAM-Ala-D-Glu-NH_2 | $NH-(CH_2)_3-CH_3$	200	13.3 ± 0.9*
FIA + NAM-Ala-D-Glu-NH_2 | $NH-(CH_2)_{17}-CH_3$	200	6.3 ± 1.6
FIA + NAM-Ala-D-Glu-NH_2	200	9.1 ± 1.1*

Values represent the means from 6–10 animals ± fiducial limits and are expressed in diameter of redness (mm). * Statistically significant results; $p < 0.05$. NAM = N-acetylated muramic acid. Unless stated otherwise, all optically active amino acids are of L-configuration.