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Immunité du lapin contre la tique *Ixodes ricinus* L. (Ixodoidea, Ixodidae): mécanismes effecteurs et leurs effets sur la biologie de l'ectoparasite.

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IMPRIMATUR POUR LA THÈSE

Immunité du lapin contre la tique Ixodes ricinus L. (Ixodoidea, Ixodidae): mécanismes effecteurs et leurs effets sur la biologie de l'ectoparasite

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DÉVELOPPEMENT D'UNE HYPERSENSIBILITÉ RETARDÉE

chez des lapins infestés par les femelles d'*Ixodes ricinus* L.[1]

P. GIRARDIN et M. BROSSARD*

RÉSUMÉ. Au cours de 4 infestations successives par *I. ricinus* L., la peau de lapin se sensibilise progressivement aux antigènes salivaires des ectoparasites. Le phénomène est décelé par la mesure au micromètre d'un repli dermique au point d'injection de l'extrait salin antigénique.
Un léger épaississement de la peau des lapins infestés est noté durant l'heure suivant d'administration d'antigène. Il est sans doute dû à une faible hypersensibilité cutanée de type immédiat. L'épaississement est maximal après 24 à 48 h et s'amplifie au cours des infestations. Cette réaction tardive est certainement l'expression d'une hypersensibilité de type retardé (type IV = tuberculinique classique).

Development of delayed-type hypersensitivity by rabbits infested with *Ixodes ricinus* L. females

SUMMARY. During the course of 4 successive infestations with *I. ricinus* L., the skin of rabbits became progressively more sensitive to the salivary antigens from the ectoparasites. The phenomenon was evaluated by measuring a skin fold at the point of injection of the antigenic saline extract.
A slight thickening of the skin of infested rabbits was observed during the hour following application of antigen. It is without doubt due to a weak cutaneous immediate type hypersensitivity. The skin thickness was at a maximum after 24 to 48 hrs and increased with each infestation. This late reaction is certainly the expression of a delayed-type hypersensitivity (type IV = classical tuberculin reaction).

I — Introduction

Des lapins acquièrent progressivement une résistance contre la piqûre des femelles d'*Ixodes ricinus* L. au cours de 4 infestations successives (Bowessidjaou et coll., 1977 ; Brossard et coll., 1982). Ce phénomène affecte la biologie des ectoparasites. La durée moyenne du repas sanguin est prolongée, la prise de sang et le rendement des pontes sont diminués. La résistance perturbe également l'embryogenèse. Cette immu-

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nité peut être partiellement transférée au moyen d'immunsérum d'animaux pluri-infestés (Brossard, 1977 ; Brossard et Girardin, 1979).

Le site de fixation des tiques dans la peau des lapins est fortement infiltré par des cellules mononucléées et polynucléées (Brossard et Fivaz, 1982). Ainsi, des basophiles affluent et dégranulent en plus grand nombre au cours d'une réinfestation que lors d'une primo-infestation des animaux. Cette réaction est similaire à une anaphylaxie cutanée à basophiles. Ces leucocytes sont en effet sensibilisés contre les antigènes salivaires de tiques, phénomène démontré par un test de dégranulation *in vitro* (Brossard et coll., 1982). Les éosinophiles sont mobilisés plus précocement et aussi de manière plus intense, tandis que neutrophiles et lymphocytes envahissent le tissu massivement quelle que soit l'infestation. Les mastocytes dégranulent également en plus forte proportion au cours d'une réinfestation. Cette dernière observation caractérise sans doute le développement d'une hypersensibilité de type immédiat.

Les effets de l'immunité des lapins sur la biologie des tiques sont diminués par l'injection quotidienne de mépyramine, un anti-histaminique-H1 (Brossard, 1982). Cette expérience démontre l'importance de l'histamine dans l'expression de la résistance.

L'étude présente montre que des lapins infestés par *I. ricinus* développent de surcroît une hypersensibilité de type retardé. Au cours de 4 infestations successives des animaux, le phénoméne évolue progressivement.

II — Matériel et méthodes

2.1 Lapins

Cinquante-cinq lapins (10 par infestation et 15 contrôles) de souche Himalayenne et de génotype aac^{H}c^{H} ont été utilisés. Lors des expériences, ils étaient âgés de 3 mois (contrôles et première infestation) à 6 mois et demi (4^{e} infestation) et pesaient 2,5 kg en moyenne. Ces animaux ont été infestés par 10 femelles et 10 mâles d'*I. ricinus*. Seules les femelles de cette espèce se gorgent, mais la copulation est nécessaire à une prise de sang normale (Graf, 1978a). L'essai cutané a été réalisé 15 jours après la fin de chaque infestation *(fig. 1)*. Le même intervalle a été respecté avant de réinfester les animaux des autres lots.

2.2 Tiques

Les tiques *I. ricinus* utilisées pour les infestations et la préparation d'antigène sont élevées dans notre laboratoire selon la méthode de Graf (1978b). Lors de l'infestation des lapins, les tiques sont déposées dans un fourreau de drap fixé à la base de l'oreille de l'hôte et fermé à son extrémité distale au moyen de toile adhésive. Un large collier empêche les lapins de se défaire des parasites par grattage.

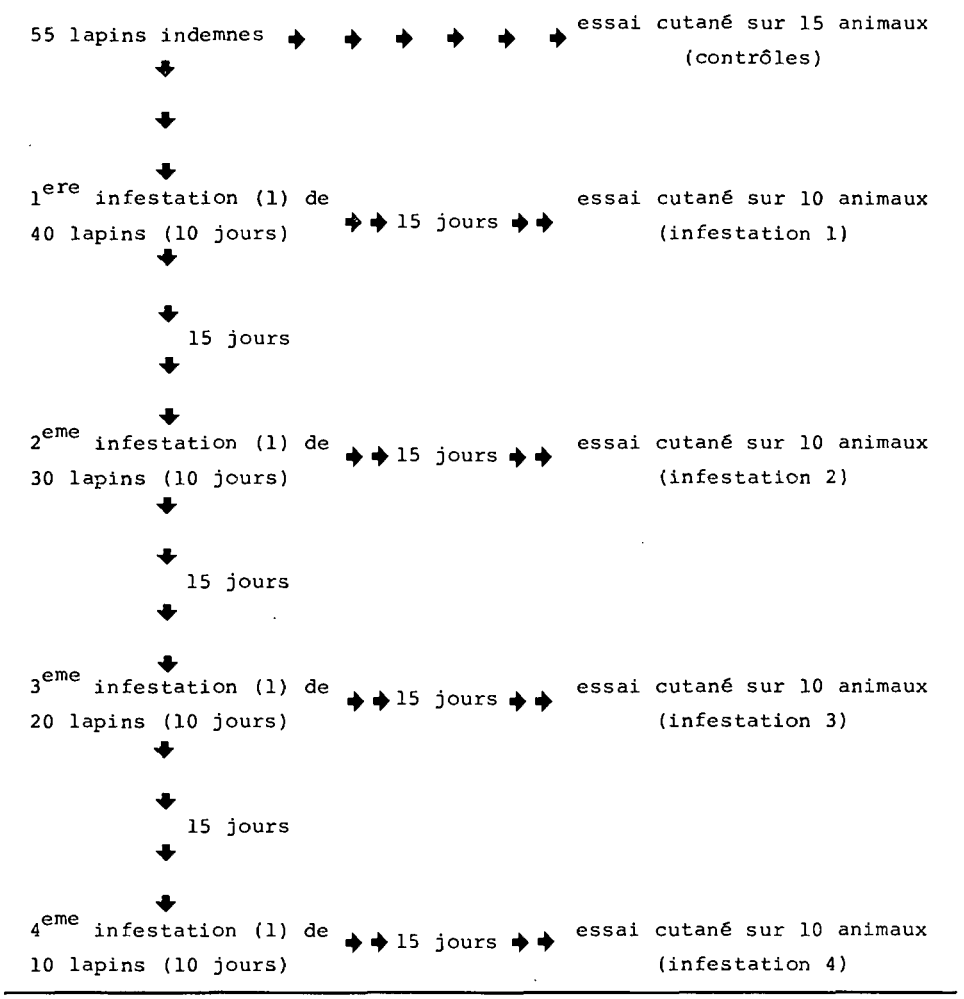

FIG. 1. — Schéma expérimental.

(1) : 10 ♀♀ et 10 ♂♂ d'*I. ricinus*.

2.3 Antigène

Après 4 jours de nutrition sur lapin, des femelles d'*I. ricinus* sont disséquées. Les glandes salivaires sont prélevées, lavées, puis broyées dans du tampon phosphate 0,01 M à pH = 7,2 maintenu à 4° C. Le broyat est centrifugé pendant 30' à 10 000 G et 4° C. Le surnageant est dialysé pendant 24 h (membrane sélective pour des PM > 7-8 000) à 4° C contre le même tampon. Les protéines du dialysat sont dosées selon la méthode de Lowry et coll. (1951). L'antigène est conservé lyophilisé.

2.4 Mesure de la réaction cutanée

Toutes les 10 minutes pendant l'heure suivant les injections d'antigène, ainsi qu'après 6, 24, 48, 72 et 96 h, la réaction cutanée est évaluée par la mesure au micromètre de l'épaisseur d'un repli de la peau des lapins. Le ventre des animaux est rasé 24 h avant le début des expériences. Les mesures sont effectuées au centre de 3 disques d'un diamètre approximatif de 1 cm, dessinés avec un stylo feutre, et distants d'au moins 5 cm. Deux surfaces servent de contrôle : l'influence de l'injection de 50 µl de tampon phosphate 0,01 M à pH = 7,2 et du pincement de la peau par le micromètre est estimée respectivement dans les disques 1 et 3. Le troisième endroit (disque 2) est réservé à l'administration de l'antigène. La dose antigénique optimale a été expérimentalement fixée à 50 µg dans 50 µl de tampon phosphate 0,01 M à pH = 7,2. Les essais préliminaires avaient été effectués sur des lapins immuns avec les doses respectives de 5, 10, 20, 50, 100 et 200 µg.

2.5 Traitement des résultats

Une statistique globale des données a été réalisée pour chaque groupe de lapins (contrôles et infestations 1 à 4). Il a ensuite été procédé à des tests de Student pour détecter des différences d'épaississement significatives :

— entre les trois disques choisis,

— entre les intervalles de mesure (cinétique de la réaction),

— entre les différentes infestations, au point d'injection de l'antigène.

III — Résultats

3.1 Remarques préliminaires

Le diamètre des indurations apparues au seul point d'injection de l'antigène chez les lapins infestés n'a pas été pris en considération. Cette mesure est trop imprécise et de grandes variations sont enregistrées entre animaux d'un même groupe. L'expression des épaississements de la peau en pourcents ou en quotients par rapport aux épaisseurs initiales a été écartée : quels que soient les surfaces ou groupes de lapins choisis, l'accroissement dermique n'a jamais été, pour un intervalle de temps fixé, proportionnel à l'épaisseur initiale.

3.2 Statistique globale *(tableau I)*

Chez les lapins contrôles, on note un léger accroissement moyen de la peau, maximal et non spécifique après 30′ dans le disque 1 (injection du solvant), 24 h dans le disque 2 (injection de l'antigène) et 50′ à 6 h dans le disque 3 (aucune injection). Il reste borné à des valeurs très modestes (respectivement 0,28 mm, 0,45 mm et 0,16 mm).

TABLEAU I. — Statistique globale.

SITE	TEMPS	Contrôles		Infest. 1		Infest. 2		Infest. 3		Infest. 4	
		MOY.	E.-T.	MOY.	E.-T.	MOY.	E.-T.	MOY.	E.-T.	MOY.	E.-T.
1	10'	18	8	24	14	18	15	29	24	17	11
1	20'	22	12	26	16	32	25	21	20	20	14
1	30'	28	15	27	23	30	20	26	23	19	15
1	40'	27	16	25	20	27	22	28	23	21	15
1	50'	23	15	21	23	32	25	25	23	19	11
1	60'	23	16	22	20	31	26	24	19	20	11
1	6 h	21	15	15	13	29	31	23	17	20	12
1	24 h	14	10	29	25	26	36	13	10	17	13
1	48 h	10	14	22	23	12	13	12	6	15	12
1	72 h	8	10	19	24	11	18	7	6	7	4
1	96 h	4	4	15	25	16	29	4	3	6	6
2	10'	24	19	27	17	10	8	29	21	26	18
2	20'	33	29	37	17	26	17	36	24	37	26
2	30'	43	39	39	21	38	21	45	33	40	26
2	40'	43	39	36	15	37	24	61	43	50	28
2	50'	39	30	31	16	37	19	48	35	47	25
2	60'	39	28	33	20	35	15	48	36	49	28
2	6 h	34	26	64	25	80	22	100	55	113	43
2	24 h	45	30	106	54	213	64	269	166	224	62
2	48 h	38	43	87	44	212	93	302	144	242	66
2	72 h	25	32	74	43	188	112	209	99	192	63
2	96 h	24	32	43	22	130	96	151	106	133	60
3	10'	9	9	13	12	3	3	9	10	6	4
3	20'	10	10	13	15	6	5	10	10	12	7
3	30'	12	11	18	20	8	7	15	12	10	10
3	40'	13	11	21	22	13	9	17	10	13	8
3	50'	16	13	21	25	13	9	17	9	17	11
3	60'	16	14	23	22	13	11	17	10	17	13
3	6 h	16	12	19	18	13	9	17	7	11	8
3	24 h	9	6	23	13	15	14	10	9	17	12
3	48 h	4	4	15	12	10	13	17	11	21	20
3	72 h	5	4	11	8	6	3	11	9	14	9
3	96 h	3	2	6	5	6	10	7	8	7	7

Contrôles : n = 15
Infestations 1-4 : n = 10
MOY. : moyennes (centièmes de mm)
E.-T. : écarts-types (centièmes de mm)

Chez les animaux infestés, aucun accroissement notable n'est également décelé aux deux sites témoins (1 et 3), les épaississements maximaux relevés étant respectivement de 0,32 mm (infestation 2, site 1) et de 0,23 mm (infestation 1, site 3).

Au site d'injection de l'antigène (disque 2), l'épaississement maximal moyen apparaît au bout de 24 h chez les lapins infestés 1 fois (1,06 mm) et 2 fois (2,13 mm).

C'est chez les lapins ayant subi 3 infestations que l'accroissement moyen d'un repli dermique est le plus élevé (3,02 mm après 48 h).

Enfin, après 4 infestations des lapins, la mesure maximale est obtenue après 48 h (2,42 mm). Elle avoisine celle relevée chez les lapins infestés à deux reprises.

3.3 Comparaison des épaississements du repli de la peau entre les trois différents disques *(tableau I)*

a) *Comparaison entre les sites 1* (injection du solvant) *et 3* (indemne) :

Lors des infestations 1 et 3, aucune différence significative d'épaississement de la peau n'est relevée pour tout moment d'observation. Dans les autres cas, on note quelques différences significatives ($p < 0{,}01$) : lors de la première mesure (après 10') pour l'infestation 4 et pendant les 30 ou 40 premières minutes de l'expérience (respectivement infestation 2 et contrôles). Ces différences sont ainsi observées exclusivement au début de l'essai. Les épaississements sont toujours supérieurs au site 1. La lente résorption du solvant en est sans doute responsable. Ainsi les sites 1 (injection du solvant) et 3 (permettant de mesurer l'influence seule du pincement) se comportent de manière très comparable, exception faite de la période nécessaire à la résorption du solvant (10-40'). Par conséquent, nous négligerons dans la suite de l'étude le site 3 et ne considérerons que le site 1 comme disque contrôle.

b) *Comparaison entre les disques 1* (injection du solvant) *et 2* (administration de l'antigène) :

Lors d'une primo-infestation, on constate un épaississement significativement supérieur au site 2 entre 6 et 72 h après les injections ($p < 0{,}01$). En revanche, un écart n'est noté qu'après 24 h dans le lot des lapins contrôles. Dès la deuxième infestation, on décèle une différence significative continue dès 6 h après la stimulation antigénique (4e infestation dès 40' déjà).

3.4 Évolution de l'épaississement de la peau au site 2 (injection d'antigène

Les valeurs relevées au cours de l'expérience ont été confrontées entre elles pour chaque infestation (évolution significative de la réponse immunitaire) *(tableau II et figure 2)*.

TABLEAU II. — Test de Student sur les différences d'épaisseur de la peau dans le disque 2.

	CONTRÔLES										
	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10	T11
T1											
T2											
T3											
T4											
T5											
T6											
T7											
T8	*										
T9											
T10											
T11											

TABLEAU 11 *(suite)*.

INFESTATION 1

	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10	T11
T1											
T2											
T3											
T4											
T5											
T6											
T7	***	**	*	**	**	**					
T8	***	***	***	***	***	***	*				
T9	***	**	**	***	**	**					
T10	**	*	*	*	**	*					
T11								**	*		

INFESTATION 2

	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10	T11
T1											
T2	*										
T3	***										
T4	**										
T5	***										
T6	***										
T7	***	***	***	***	***	***					
T8	***	***	***	***	***	***	***				
T9	***	***	***	***	***	***	***				
T10	***	***	***	***	***	***	**				
T11	***	**	**	**	**	**		*			

INFESTATION 3

	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10	T11
T1											
T2											
T3											
T4	*										
T5											
T6											
T7	***	**	*		*	*					
T8	***	***	***	**	***	***	**				
T9	***	***	***	***	***	***	***				
T10	***	***	***	***	***	***	**				
T11	**	**	**	*	**	**			*		

INFESTATION 4

	T1	T2	T3	T4	T5	T6	T7	T8	T9	T10	T11
T1											
T2											
T3											
T4	*										
T5	*										
T6	*										
T7	***	***	***	***	***	***					
T8	***	***	***	***	***	***	***				
T9	***	***	***	***	***	***	***				
T10	***	***	***	***	***	***	**				
T11	***	***	***	***	***	***		**	***	*	

* prob < 0.05
** prob < 0.01
*** prob < 0.001

T1 = 10′
T2 = 20′
T3 = 30′
T4 = 40′
T5 = 50′
T6 = 60′

T7 = 6 h
T8 = 24 h
T9 = 48 h
T10 = 72 h
T11 = 96 h

a) *Contrôles.* Le repli dermique n'est significativement épaissi ($p < 0,05$) qu'après 24 h, et seulement par rapport à la valeur obtenue après 10'.

b) *Infestation 1.* Durant la période comprise entre 6 et 72 h après l'injection d'antigène, les épaississements de la peau sont significativement différents de ceux observés antérieurement ($p < 0,05$). On constate, après 24 h, une forte augmentation de l'épaisseur du derme, puis un retour progressif à l'état antérieur.

c) *Infestation 2.* Toutes les épaisseurs mesurées lors de cette infestation diffèrent de celle relevée 10' après l'administration d'antigène ($p < 0,05$). Les valeurs de la première heure sont en outre inférieures à celles mesurées ultérieurement ($p < 0,01$). Les épaississements notés après 6, 48 et 72 h ne sont pas dissemblables de celui obtenu après 96 h.

d) *Infestation 3.* Durant la première heure d'observation, un seul épaississement dermique est supérieur à la première mesure (40', $p < 0,05$). Les épaississements diffèrent, durant cette période, de ceux mesurés ultérieurement ($p < 0,05$). On constate cependant une exception : les valeurs relevées après 40' et 6 h ne sont pas significativement différentes entre elles. De même, aucune différence n'est relevée entre la dernière valeur et les mesures effectuées 6, 24 et 72 h après les injections.

e) *Infestation 4.* De la 40e à la 60e minute, l'épaississement de la peau des lapins est supérieur à celui mesuré après 10' ($p < 0,05$). Dès lors, il s'accroît sensiblement ($p < 0,01$). Enfin, lors de la dernière mesure, l'épaisseur du repli dermique est proche de celle relevée après 6 h.

3.5 Comparaisons, au site 2, entre les infestations *(fig. 2)*

a) *Remarques générales.* Pendant la première heure d'observation, on ne décèle aucune différence significative entre les divers groupes de lapins, sauf lors de la première mesure. En effet, les animaux infestés 2 fois présentent à ce moment une réaction significativement inférieure à celle des contrôles et des animaux primo-infestés ($p < 0,05$). Cette différence est sans doute essentiellement imputable à la dispersion des mesures.

b) *Comparaisons entre contrôles et sujets infestés.* A l'exception de la 1re infestation, les mesures effectuées sur les lapins infestés diffèrent ($p < 0,01$) de celles relevées chez les lapins indemnes entre 6 et 96 h après l'administration d'antigène. En effet, les lapins primo-infestés ne montrent une différence qu'après 6, 24 et 72 h, par rapport aux lapins non infestés ($p < 0,01$).

c) *Comparaisons entre les réactions cutanées des lapins primo-infestés et pluri-infestés.* De la 24e à la 72e heure d'observation, les réactions des lapins primo-infestés diffèrent de celles des animaux infestés 2 fois ($p < 0,01$). Dès 24 h, elles sont également inférieures à celles des individus infestés 3 fois ($p < 0,01$). Enfin, toutes les réactions cutanées observées dès la 6e heure sont dissemblables chez des lapins infestés 1 ou 4 fois ($p < 0,005$).

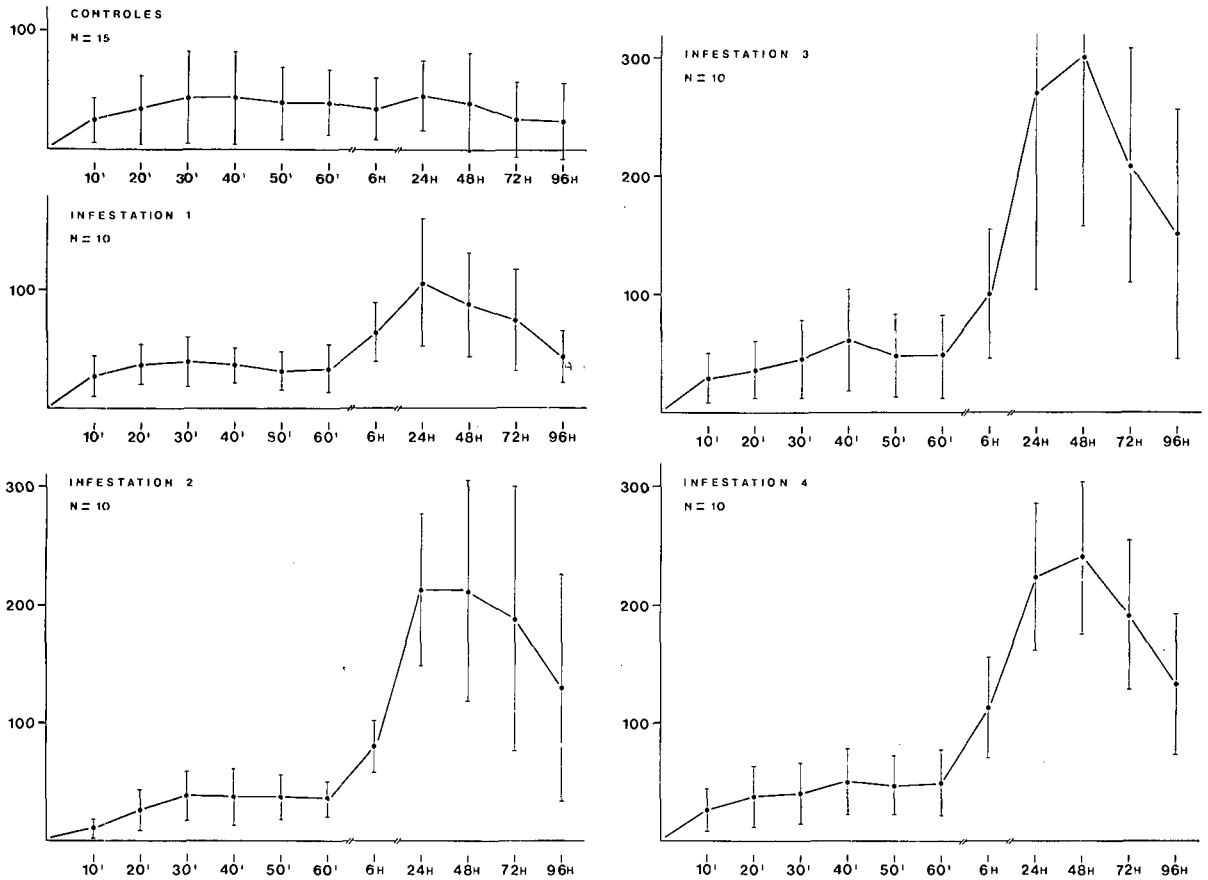

FIG. 2. — Évolution de l'épaississement de la peau des lapins au site d'injection de l'antigène (moyenne ± écart-type).

d) *Conclusion.* Les réactions des contrôles diffèrent de celles de tous les autres groupes de lapins. En outre, les animaux primo-infestés développent une réponse inférieure à celle des sujets pluri-infestés. Enfin, les résultats obtenus chez des lapins ayant nourri des tiques 2, 3 ou 4 fois ne diffèrent pas statistiquement.

IV — Discussion

Dans notre expérience, la peau des lapins s'épaissit légèrement pendant l'heure suivant l'injection intradermique d'antigène (extrait salivaire de tique). Seuls les animaux infestés 3 fois par *I. ricinus* montrent alors un épaississement significativement supérieur à la première mesure ($p < 0{,}05$), ceci après 40'. Ce phénomène est sans doute la manifestation d'une hypersensibilité de type immédiat. Brossard et

Fivaz (1982) ont observé des mastocytes dégranulés au point de fixation des ectoparasites, en plus grand nombre sur des lapins réinfestés. En outre, des éosinophiles envahissent plus précocement et plus massivement le tissu. Par un test de Präusnitz-Küstner, Brossard et Girardin (1979) ont décelé des anticorps homocytotropes dans le sérum d'animaux infestés. La peau de receveurs a été sensibilisée (type immédiat) par l'injection intraveineuse de ces immunsérums. Des bovins infestés par *Boophilus microplus* développent aussi une hypersensibilité cutanée de type immédiat (Willadsen et coll., 1978). Wikel et coll. (1978) détectent également une légère réaction cutanée immédiate chez des cobayes résistants soumis à une injection intradermique d'antigène salivaire de *Dermacentor andersoni*. Ils imputent plutôt ce phénomène à l'effet d'agents salivaires vasoactifs.

Dès 6 h après la stimulation antigénique, l'épaississement de la peau de lapins infestés est plus marqué. On observe alors l'apparition d'un érythème. La réaction atteint son apogée après 24 à 48 h et est accompagnée d'une induration.

Dans le lot des contrôles, nous notons, après 24 h, un épaississement dermique supérieur ($p < 0,05$) à celui obtenu après 10'. Cependant, cette réaction n'est pas comparable à celle des animaux infestés ($p < 0,01$). Elle est sans doute non spécifique, car nous n'avons pas observé d'érythème ou d'induration.

Les lapins primo-infestés réagissent différemment des contrôles et des animaux pluri-infestés. En revanche, aucune distinction significative ne peut être décelée entre les réactions des sujets infestés 2, 3 ou 4 fois. Les effets de la résistance sur la biologie des femelles d'*I. ricinus* sont également plus marqués au fil des infestations et plafonnent dès la 3e (Bowessidjaou et coll., 1977 ; Brossard et coll., 1982). Les basophiles se sensibilisent aussi plus fortement aux antigènes salivaires après une 2e infestation (Brossard et coll., 1982). Ils dégranulent dans une proportion sensiblement égale lors d'infestations ultérieures. La production d'anticorps anti-tique de la classe IgG augmente chez les lapins réinfestés et leur titre est maximal après 3 (Bowessidjaou et coll., 1977) ou 4 infestations (Brossard et coll., 1982).

Ces différents phénomènes peuvent intervenir dans l'établissement d'une immunité. Nous avions démontré que des facteurs humoraux (sans doute des anticorps IgG et IgE) ne conféraient qu'une immunité partielle à des receveurs d'immunsérum (Brossard, 1977 ; Brossard et Girardin, 1979).

Ainsi, dans le présent travail, nous avons montré que les lapins soumis à des infestations successives d'*I. ricinus* développent une hypersensibilité de type immédiat et une hypersensibilité de type retardé. D'après Gell et Coombs (1968), cette dernière est une réaction tuberculinique de type classique (type IV). Révélée par un essai cutané, elle est en effet encore présente un mois après le début des infestations. Elle est maximale 24 à 48 h après la stimulation antigénique et déjà fortement atténuée au bout de 96 h.

BIBLIOGRAPHIE

BOWESSIDJAOU J., BROSSARD M., AESCHLIMANN A. : Effect and duration of resistance acquired by rabbits on feeding and egg laying in *Ixodes ricinus* L. *Experientia*, 1977, *33*, 528-530.

BROSSARD M. : Rabbits infested with the adults of *Ixodes ricinus* L. : passive transfer of resistance with immune serum. *Bull. Soc. Pathol. Exot.*, 1977, *70*, 289-294.

BROSSARD M., GIRARDIN P. : Passive transfer of resistance in rabbits infested with adults *Ixodes ricinus* L. : humoral factors influence feeding and egg laying. *Experientia*, 1979, *35*, 1395-1396.

BROSSARD M. : Rabbits infested with adult *Ixodes ricinus* L. : Effects of mepyramine on acquired resistance. *Experientia*, 1982, *38*, 702-703.

BROSSARD M., FIVAZ V. : *Ixodes ricinus* L. : Mast cells, basophils and eosinophils in the sequence of cellular events in the skin of infested or re-infested rabbits. *Parasitology*, 1982, *85*, 583-592.

BROSSARD M., MONNERON J.-P., PAPATHEODOROU V. : Progressive sensitization of circulating basophils against *Ixodes ricinus* L. antigens during repeated infestations of rabbits. *Parasite Immunol.*, 1982, *4*, 355-361.

GRAF J.-F. : Copulation, nutrition et ponte chez *Ixodes ricinus* L. (Ixodoidea : Ixodidae)-3e partie. *Bull. Soc. Entomol. Suisse*, 1978a, *51*, 343-360.

GRAF J.-F. : Copulation, nutrition et ponte chez *Ixodes ricinus* L. (Ixodoidea : Ixodidae) - 1re partie. *Bull. Soc. Entomol. Suisse*, 1978b, *51*, 89-97.

GELL P. G. H., COOMBS R. R. A. : Classification of allergic reactions responsible for clinical hypersensitivity and disease. *In :* Gell P. G. H. et Coombs R. R. A. Eds, *Clinical aspects of immunology*, 2e édition, Blackwell, Oxford, 1968, p. 575.

LOWRY O. H., ROSEBROUGH N. J., FARR A. L., RANDALL R. J. : Protein measurement with the Folin Phenol Reagent. *J. Biol. Chem.*, 1951, *193*, 265.

WIKEL S. K., GRAHAM J. H., ALLEN J. R. : Acquired resistance to ticks : IV. Skin reactivity and *in vitro* lymphocyte responsiveness to salivary gland antigen. *Immunology*, 1978, *34*, 257-263.

WILLADSEN P., WILLIAMS P. E., ROBERTS J. E., KERR J. D. : Response of cattle to allergens from *Boophilus microplus*. *Int. J. Parasitol.*, 1978, *8*, 89-95.

Parasitol Res (1989) 75:657–662

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Effects of cyclosporin A on humoral immunity to ticks and on cutaneous immediate and delayed hypersensitivity reactions to *Ixodes ricinus* L. salivary-gland antigens in re-infested rabbits

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Abstract. When rabbits are repeatedly infested with *Ixodes ricinus* L. adults, they acquire resistance to these ticks. A humoral response occurs and the skin of the host becomes progressively sensitized to the saliva of the ectoparasites. The present study examined the effects of cyclosporin A on these two aspects of immunity in re-infested rabbits. Using the enzyme-linked immunosorbent assay (ELISA), moderate suppression of the secondary IgG response to bites of these ticks was observed in animals given this immunosuppressive drug. The agent blocked the immediate cutaneous (type I) reaction normally developed in response to the intradermal injection of *I. ricinus* salivary gland antigens, as detected by measuring the skinfold thickness. The same method revealed a decreased delayed (type IV) hypersensitivity to these antigens. The present experiments demonstrate the major role of cyclosporin A-sensitive cells (mostly T-lymphocytes, mast cells and basophils) involved in the complex phenomenon of resistance to ticks.

Rabbits progressively acquire resistance to the bites of *Ixodes ricinus* L. females (Bowessidjaou et al. 1977; Brossard et al. 1982). The formation of anti-salivary-gland IgG (Brossard 1977; Brossard and Girardin 1979) and IgE (Brossard and Girardin 1979) antibodies has been demonstrated by indirect immunofluorescence and passive cutaneous anaphylaxis tests. Furthermore, acquired resistance has been partially transferred by means of immune sera from multi-infested animals (Brossard 1977; Brossard and Girardin 1979). The fixation site of the ticks in the skin of such rabbits is heavily infiltrated by mono- and polynucleate cells (Brossard and Fivaz 1982), degranulated mast cells and basophils, which occur in higher numbers in re-infested animals. Circulating basophils progressively become sensitive to *I. ricinus* salivary gland antigens, as previously demonstrated by an in vitro degranulation test (Brossard et al. 1982). The locally liberated histamine probably plays a role in the expression of resistance; in fact, the daily injection of the antihistaminic H-1 agent mepyramine diminishes the effects of host immunity on the biology of the ectoparasites (Brossard 1982). Although humoral immunity is certainly important in the rabbits' acquisition of resistance to *I. ricinus* females, the role of cell-mediated immunity cannot be excluded. Thus, Girardin and Brossard (1985) have described the development of a delayed-type hypersensitivity in multi-infested rabbits.

To define better the immunological basis of the rabbits' resistance to ticks, these hosts have been treated with the immunosuppressive agent cyclosporin A during repeated infestations (Girardin 1986). Cyclosporin A may act in two ways on the biology of the ticks: (1) by inhibiting the immune response of the host, thus enabling the female ticks to take a larger bloodmeal and lay more eggs; and (2) by diminishing the egg-conversion factor of the ectoparasites (weight of eggs laid/weight of the engorged female), hence provoking failures in oviposition and hatching.

In the present study, the relative importance of the humoral and cellular compartments in the establishment of rabbits' immunity against ticks

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was better determined. We evaluated the effects of treatment with cyclosporin A on the immune response of rabbits naturally sensitized to salivary-gland antigens of *I. ricinus*. The influence of this drug on the secondary humoral response (during a second infestation) and the immediate and delayed cutaneous reactions (normally developed after a re-infestation) were examined.

Material and methods

Rabbits. Male Himalayan rabbits of the genotype aac^Hc^H were used. These animals were 2–2.5 months old and weighed approximately 2 kg at the beginning of the experiments. Each rabbit was subjected to two infestations with ten female and ten male of *I. ricinus*, first on day 0 and then on day 23 of the study. Only females of this species engorge, but copulation is necessary for an optimal bloodmeal (Graf 1978a).

Ticks. The *I. ricinus* ticks used for the two infestations of the rabbits and for the salivary-gland antigen preparations were reared in our laboratory according to the method of Graf (1978b). During infestations, the ticks were confined in a cloth bag placed over the ear of the host; a collar prevented grooming.

Treatment with cyclosporin A. As described by Lindsey et al. (1980), cyclosporin A (Sandoz S.A., Basel) was dissolved in mineral oil (Miglyol 812, Dynamit Nobel); a daily dose of 25 mg/kg was given subcutaneously to the host animals. The six rabbits in group 1 (control group) were subjected to an injection of the solvent (only mineral oil) on days −3–+4 of the second infestation. Cyclosporin A was given to the six animals in group 2 on days −1,0 and +1 of the cutaneous test and to the five rabbits in group 3 during the re-infestation (days −3–+4).

Antigen. After feeding for 4 days on rabbits, *I. ricinus* females were dissected. All preparation steps were carried out at 4° C. The salivary glands were removed, washed, and homogenized in phosphate-buffered saline (PBS; 0.02*M* PO_4^-, 0.03*M* NaCl, pH 7.2). The homogenate was centrifuged for 30 min at 10000 *g* and the supernatant was dialyzed for 24 h (membrane selective for a mol. wt. of >7–8 kDa) against the same buffer. The protein concentration of the dialyzed sample was measured using the method of Lowry et al. (1951). The antigen was lyophilized; once reconstituted, it was used either for cutaneous tests or for the titration of the circulating specific IgG by enzyme-linked immunosorbent assay (ELISA).

Cutaneous test. The cutaneous test was conducted 12 days after the end of the second infestation. The cutaneous reaction was evaluated every 10 min for the 1st h following the intradermal injections and after 6, 24, 48 and 96 h by measuring the thickness of the rabbit's skin with a micrometer. The abdomen of the animals was shaved 24 h before the beginning of the test. Measurements were taken at the center of two 1-cm diameter rings previously drawn with a felt-tip pen and at least 5 cm apart. One surface served as the control (disc 1), where the effect of the solvent (50 µl PBS) was estimated. The second place (disc 2) was used for the inoculation of the antigen. The optimal antigen dose had previously been determined as 50 µg (Girardin and Brossard 1985).

Titration of anti-salivary-gland antibodies. On days 2, 4, 6, 9, 12, 17, 25, 27, 29, 31, 34 and 39 of the experiment, 2 ml blood was drawn from the central artery of one ear of each animal. The antibody titres of the sera were estimated by ELISA. The method described by Martinod et al. (1985) was adapted to our experimental conditions. Peroxidase protein A was replaced by horseradish peroxidase conjugated to goat IgG anti-rabbit IgG (American Qualex) diluted 5000 times. A pool of sera from rabbits infested four times and from non-immune animals was used to test the specificity of the reaction. The microplates (Dynatech) had previously been coated with the salivary-antigen preparation (0.5 µg/well, diluted in 100 µl 50 m*M* carbonate buffer, pH 9.6).

Statistics. Both studied variables (skin thickening and antibody titres) had a Gaussian distribution. Student's *t*-test was therefore used to detect significant differences in the cutaneous thickening between (1) the two discs (buffer alone or with antigen), (2) the two measurements (kinetics of the reactions) and (3) the three groups of rabbits. The same test was used to detect differences in the specific antibody titres between the blood samples (kinetics of the antibody production) as well as between controls and treated rabbits.

Results

Antibody production

Kinetics of antibody production. None of the rabbits used in this study synthesized anti-*I. ricinus* salivary-gland antigen IgG in quantities detectable by ELISA before the 12th day following the beginning of the first infestation (Fig. 1). A significant production of antibody ($P<0.05$) was observed only on day 17 in the 12 control rabbits (groups 1 and 2 together) and day 25 in the 5 treated animals (group 3). The antibody titres of the control rabbits increased progressively from the 27th day of the experiment (4 days after the fixation of the ticks for re-infestation; $P<0.01$–$P<0.001$) and reached their maximum after 34 days, after which they declined, becoming significantly lower ($P<0.05$) 5 days later. In treated rabbits, antibody produc-

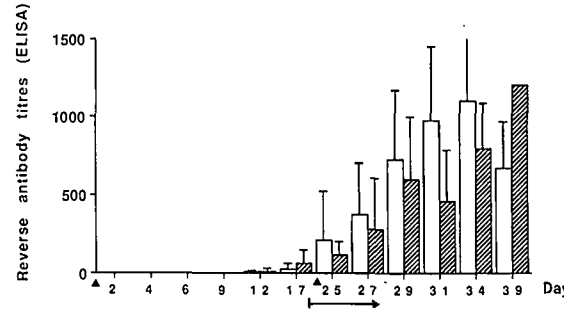

Fig. 1. Production of IgG specific to *I. ricinus* salivary-gland antigen in the course of two successive infestations. ▲, infestation with 10 female and 10 male *I. ricinus*; →, daily treatment with cyclosporin A (25 mg/kg). □, controls ($n=12$); ▨, treated animals ($n=5$)

tion continued to increase significantly between the 25th and 29th days of the experiment ($P<0.05$). This production was diminished on day 31 ($P<0.05$), but on day 34 (7 days after the end of the cyclosporin A treatment) an increase in antibody synthesis was again observed. The final measurement (day 39) gave the highest titre ($P<0.05$–$P<0.01$ relative to the former values).

Comparisons between control and treated rabbits. The rabbits treated with cyclosporin A produced less IgG specific to *I. ricinus* salivary-gland antigens than controls. However, this difference was significant only on the 31st day of the experiment ($P<0.01$). On the other hand, at the end of the experiment (8 days after the 2nd lot of ticks had dropped), the synthesis of specific antibodies had decreased in control rabbits but reached its highest value in treated animals ($P<0.01$).

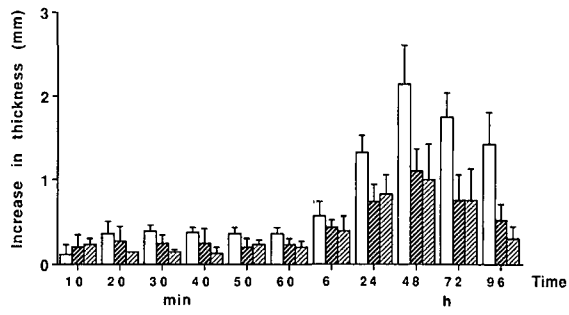

Fig. 2. Cutaneous reactions of rabbits re-infested with *I. ricinus* adults to the intradermal injection of tick salivary gland antigen (50 µg in 50 µl PBS). The 6 control rabbits (group 1) were subjected to an injection of the solvent for cyclosporin A (Miglyol 812, Dynamit Nobel) on days −3–+4 of the second infestation. Cyclosporin A (25 mg/kg) was given to the 6 animals in group 2 on days −1–+1 of the skin test, whereas the immunosuppressive solution was injected into the 5 rabbits in group 3 during the second infestation (days −3–+4). □, group 1; ▨, group 2; ▩, group 3

Cutaneous reactions

Comparison of the thickness of skin injected with PBS vs antigen. In the three groups of rabbits, few changes in the skin's thickness were observed in control disc 1 (buffer injected alone; results not shown). A statistical comparison of the values recorded at each time in the two discs indicated large differences in the evolution of skin thickness between the two measurement sites. The skin of the control animals was thicker in disc 2 (antigen inoculation) throughout the experimental period, beginning as early as 20 min after the injection ($P<0.05$–$P<0.001$). In group 2 rabbits (treated during the skin test) this difference was slightly delayed, a significantly higher thickening in disc 2 being detected 6 h after the antigenic stimulation ($P<0.001$). The administration of the immunosuppressive drug during the second infestation (group 3) caused a very late manifestation of the specific cutaneous reaction (24 h after the beginning of the experiment; $P<0.05$–$P<0.01$).

Specific evolution of the thickness of the skin. For each group of rabbits, the values recorded in disc 2 (antigen administration) during the experiment were compared to determine the significance of the evolution of the cutaneous immune response (Fig. 2). During the 1st h of the experiment, the skin thickness in control rabbits increased from 20 min after the injection of the *I. ricinus* salivary-gland antigens ($P<0.01$–$P<0.001$) and was maximal 10 min later. After 6 h, a more marked thickening was detected ($P<0.05$), which increased rapidly from the 24th h following the antigenic injection ($P<0.001$) and reached its peak after 48 h. After this, the skinfolds became progressively thinner.

Animals treated with cyclosporin A during the test (group 2) did not develop a significant cutaneous reaction during the 1st h following antigen administration. After 6 h, their skinfolds were thicker ($P<0.05$–$P<0.01$) than before, increasing to reach a maximum after 48 h. At the end of the experiment (96th h), the dermis recovered to the condition recorded 6 h after the antigen injection. The skin of the rabbits in group 3 (treated with cyclosporin A during the second infestation) evolved in a particular manner during the 1st h of the assay. Effectively, its thickness decreased between the 20th and 40th min (significant fall after 20 and 30 min; $P<0.05$). Compared with the 1st h of observation, the skin thickness was significantly increased only between the 24th and 72nd h after the antigenic stimulation ($P<0.05$–$P<0.01$).

Comparisons of the thickening in disc 2 (antigen injection) between the three groups of rabbits. The two groups of rabbits treated with cyclosporin A exhibited a single significant difference in their skin thickening 30 min after the antigen injection ($P<0.05$). As mentioned above, the thickness of the skin of animals treated during the second infestation decreased between the 20th and 40th min of the experiment. During the 1st h following antigen administration, skin thickening in control rabbits was continuously greater ($P<0.05$–$P<0.001$) than in animals treated during the second infestation. Such a difference was perceptible only 30, 50 and 60 min after the antigenic stimulation

($P<0.05$–$P<0.01$) between the rabbits in groups 1 (controls) and 2 (treated during the test). However, it should be pointed out that in the group 2 rabbits we detected neither a significant thickening of the skin in disc 2 nor an increased thickness of the skinfolds in comparison with those determined in the control disc. Consequently, the observed discontinuity in significance was probably due to variations in the measurements or a lack of sensitivity of the statistical test used rather than to a biological manifestation. From the 24th h of the experiment, the two groups of treated animals continuously developed a weaker cutaneous reaction than the controls, with respective probability thresholds of $P<0.01$ for group 2 and $P<0.01$–$P<0.001$ for group 3.

Discussion and conclusions

In keeping with other experiments (Brossard 1977; Brossard and Girardin 1979; Brossard and Fivaz 1982; Girardin and Brossard 1985; Girardin 1986), the aim of this study was to determine the various components implicated in the resistance of rabbits to the tick *I. ricinus* and to evaluate their relative importance in this complex mechanism.

The synthesis of antibodies specific to *I. ricinus* salivary-gland antigens in response to the bites of this species, as well as its importance in the resistance phenomena, has previously been reported (Bowessidjaou et al. 1977; Brossard 1977; Brossard and Girardin 1979). Most investigators, among them Parker et al. (1984), agree that cyclosporin A generally acts as a powerful inhibitor of the primary humoral response, even with a single administration. In contrast the drug suppresses a secondary response only if continuously maintained in the organism (Borel and Wiesinger 1977; Thomson et al. 1983b). The influence of cyclosporin A treatment on the primary and secondary humoral responses of the rabbit has previously been studied; particularly, the work of Lindsey et al. (1980) clearly demonstrates the potency of cyclosporin A (25 mg/kg given on days 0–+5 after the immunization) to inhibit the primary production of antibodies directed against horse serum albumin. However, using this regime, these authors did not observe an alteration in the secondary humoral response.

The present study was the first attempted in a host-tick system. We demonstrated that the subcutaneous injection of cyclosporin A (25 mg/kg given on days −3–+4 of a re-infestation of the rabbits) partially suppressed the production of anti-tick salivary-gland antibodies. According to the reviews of Britton and Palacios (1982) and Borel and Lafferty (1983), this drug neither acts directly on B-cells (antibody producers) nor diminishes the proliferation of suppressor or cytotoxic T-cells (if already sensitized to antigens). Moreover, Ryffel et al. (1980, 1982) have observed high-affinity receptors for cyclosporin A on mouse and human T-lymphocytes, B-lymphocytes having a very weak ability to bind this drug. The agent affects mainly the T-helper cells by blocking their activation steps (for instance, transcription of mRNA coding for the lymphokines) as well as impairing their cellular proliferation and maturation processes. Thus, the secondary humoral response is indirectly weakened, as confirmed in our host-tick system.

The development in rabbits of an immediate hypersensitivity reaction in response to *I. ricinus* salivary-gland antigens has been reported in previous papers (Brossard and Girardin 1979; Brossard 1982; Brossard and Fivaz 1982; Girardin and Brossard 1985). The data obtained in control animals in the present study are in accordance with our former findings. To our knowledge, few, if any, studies have dealt with the in vivo effects of cyclosporin A on cutaneous anaphylactic reactions.

The present experiment demonstrates that immediate hypersensitivity is no longer expressed in rabbits treated with the immunosuppressive agent. As mentioned above, the tick-specific IgG response was moderately suppressed. We assume that this was also the case for the production of IgE antibodies, which, together with the mast cells, are important for the cutaneous immediate reaction to *I. ricinus* salivary-gland antigens (Brossard and Girardin 1979; Brossard and Fivaz 1982). Therefore, we hypothesize that in our model cyclosporin A may block the development of immediate cutaneous hypersensitivity by affecting the mast cells (and undoubtedly also the basophils) in two ways: (1) indirectly, by inhibiting the production of lymphokines active on these leucocytes as shown for macrophages Borel and Lafferty 1983); and (2) directly, by impairing the liberation of histamine from these two cell types, as previously suggested by Pedersen et al. (1985).

Rabbits that have been infested with *I. ricinus* females normally develop a delayed cutaneous (type IV) hypersensitivity in response to the intradermal injection of tick salivary-gland antigens (Girardin and Brossard 1985). The subcutaneous inoculation of cyclosporin A in these animals impairs this phenomenon. The method used for immunosuppressive treatment (during the second infestation or at the time of the skin test) did not influence the effects of this agent on the magnitude of the cutaneous reactions. Although the experi-

mental scheme and the administration methods differ, our results agree with the observations of Thomson et al. (1983b, c), whose work was carried out on guinea pigs sensitized against ovalbumin or mice immunized against sheep red blood cells. Borel et al. (1977) have demonstrated the suppressive effects of the endecapeptide on the delayed hypersensitivity expressed by tuberculin-sensitive guinea pigs. The mechanism of action of cyclosporin A on delayed type hypersensitivity has been well documented, essentially by Borel et al. (1977), Alberti et al. (1981), Bunjes et al. (1981), Hess et al. (1982) and Thomson et al. (1983a, b, c). As mentioned above, this drug inhibits the production of lymphokines; hence, the recruitment and stimulation of cells competent for the delayed reaction (lymphocytes, monocytes, macrophages) are decreased.

In conclusion, the present experiments demonstrate that, under treatment with cyclosporin A, rabbits infested twice with *I. ricinus* females showed (1) a partial and reversible inhibition of the secondary humoral response, as detected by the titration of the anti-tick salivary-gland antigens by ELISA; (2) a significant blocking of the immediate cutaneous (type I) hypersensitivity normally expressed in response to the intradermal injection of the same antigens (measurable by the skinfold test); and (3) a marked decrease in the delayed cutaneous (type IV) hypersensitivity to the salivary-gland antigens, as determined using the same test. The influence of an identical immunosuppressive treatment of rabbits on the biology of the ticks (indirect and direct effects) has been evaluated in another study (Girardin 1986). The latter study and the present experiments confirm the usefulness of cyclosporin A as a powerful investigative tool, as previously mentioned by Klaus (1987) and Truffa-Bachi (1987); furthermore, they indicate the important role played by T-helper cells, lymphokines and probably by mast cells and basophils, in the complex phenomena of the resistance of rabbits to ticks.

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References

Alberti S, Boraschi D, Luini W, Tagliabue A (1981) Effects of in vitro treatments with cyclosporin-A on mouse cell-mediated immune response. Int J Immunopharmacol 3:357–364

Borel J-F, Lafferty J (1983) Cyclosporin: speculation about its mechanism of action. Transplant Proc 15:1881–1885

Borel J-F, Wiesinger D (1977) Effect of cyclosporin-A on lymphoid cells. In: Lucas DO (ed) Regulatory mechanism in lymphocyte activation. Academic, New York, pp 716–718

Borel J-F, Feurer C, Magnee L, Staehelin H (1977) Effects of the new anti-lymphocytic peptide cyclosporin A in animals. Immunology 32:1017–1025

Bowessidjaou J, Brossard M, Aeschlimann A (1977) Effect and duration of resistance acquired by rabbits on feeding and egg laying in *Ixodes ricinus*, L. Experientia 33:528–530

Britton S, Palacios R (1982) Cyclosporin A: usefulness, risks and mechanisms of action. Immunol Rev 65:5–22

Brossard M (1977) Rabbits infested with the adults of *Ixodes ricinus*, L.: passive transfer of resistance with immune serum. Bull Soc Pathol Exot 70:289–294

Brossard M (1982) Rabbits infested with adult *Ixodes ricinus*, L.: effects of mepyramine on acquired resistance. Experientia 38:702–703

Brossard M, Fivaz V (1982) *Ixodes ricinus*, L.: mast cells, basophils and eosinophils in the sequence of cellular events in the skin of infested or re-infested rabbits. Parasitology 85:583–592

Brossard M, Girardin P (1979) Passive transfer of resistance in rabbits infested with adults *Ixodes ricinus*, L.: humoral factors influence feeding and egg laying. Experientia 35:1395–1396

Brossard M, Monneron J-P, Papatheodorou V (1982) Progressive sensitization of circulating basophils against *Ixodes ricinus* L. antigens during repeated infestations of rabbits. Parasite Immunol 4:355–361

Bunjes D, Hardt C, Roellinghoff M, Wagner H (1981) Cyclosporin A mediates immunosuppression of primary cytotoxic T cell responses by impairing the release of interleukin-1 and interleukin-2. Eur J Immunol 11:657–661

Girardin P (1986) Immunité du lapin contre la tique *Ixodes ricinus* L., (Ixodoidea, Ixodidae): mécanismes effecteurs et leur effets sur la biologie de l'ectroparasite. PhD Thesis, University of Neuchatel, Switzerland.

Girardin P, Brossard M (1985) Développement d'une hypersensibilité retardée chez les lapins infestés par les femelles d'*Ixodes ricinus*, L. Ann Parasitol Hum Comp 60:299–309

Graf J-F (1978a) Copulation, nutrition et ponte chez *Ixodes ricinus*, L. (Ixodoidea: Ixodidae). 1ère partie. Bull Soc Entomol Suisse 51:89–97

Graf J-F (1978b) Copulation, nutrition et ponte chez *Ixodes ricinus*, L. (Ixodoidea: Ixodidae). 3ème partie. Bull Soc Entomol Suisse 51:343–360

Hess A, Tutschka P, Santos GW (1982) Effect of cyclosporin A on human lymphocyte responses in vitro: III. CSA inhibits the production responses but does not inhibit the response of primed lymphocytes to TCGF. J Immunol 128:355–359

Klaus GGB (1987) Cyclosporin A as a probe for different modes of lymphocyte activation. Ann Inst Pasteur Paris 138:626–628

Lindsey NJ, Harris KR, Norman HB, Smith JC, Lee HA, Slapek M (1980) The effect of cyclosporin A on the primary and secondary immune response. Transplant Proc 12:252–255

Lowry OH, Rosenbrough NJ, Farr AL, Randall RJ (1951) Protein measurement with the Folin phenol reagent. J Biol Chem 193:265–275

Martinod S, Brossard M, Moreau Y (1985) Immunity of dogs against *Babesia canis*, its vector tick *Dermacentor reticulatus*, and *Ixodes ricinus* in endemic areas. J Parasitol 71:269–273

Parker D, Droessler K, Turk JL (1984) Kinetics of the effect

of a single doese of cyclosporin A on antibody and cell mediated immune response in the guinea pig. Int J Immunopharmacol 6:67–74

Pedersen C, Permin H, Stahl S, Kov P, Norn S, Svenson M, Mosbech H, Bentzen K (1985) Inhibitory effect of cyclosporin A on histamine release from human leucocytes and rat mast cells. Allergy 40:103–107

Ryffel B, Donatsch P, Goetz U, Tschopp M (1980) Cyclosporin receptors on mouse lymphocytes. Immunology 41:913–919

Ryffel B, Goetz U, Heuberger B (1982) Cyclosporin receptors on human lymphocytes. J Immunol 129:1978–1982

Thomson AW, Moon DK, Geczy CL, Nelson DS (1983a) Cyclosporin A inhibits lymphokine production but not response of macrophages to lymphokines. Immunology 48:291–299

Thomson AW, Moon DK, Inoue Y, Geczy CL, Nelson DS (1983b) Modification of delayed-type hypersensitivity reactions to ovalbumin in cyclosporin A treated guinea pigs. Immunology 48:301–308

Thomson AW, Moon DK, Nelson DS (1983c) Suppression of delayed-type hypersensitivity reactions and lymphokine production by cyclosporin A in the mouse. Clin Exp Immunol 52:599–606

Truffa-Bachi P (1987) Cyclosporin A: a tool for dissecting the mechanisms of the immune response. Ann Inst Pasteur Paris 138:644–648

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Gibson D. I. : Some blue whale Nematodes. *Parasitology,* 1973, *67,* 7-8.

Gibson D. I., Harris E. A. : The helminth-parasites of cetaceans in the collections of the British Museum (Natural History). *Invest. Cetacea,* 1979, *10,* 309-324.

Holmes J. C. : Evolutionary relationships between parasitic helminths and their hosts. *In:* Coevolution, D. J. Futuyama and M. Slatkin (eds.). *Sinauer Associates,* Sunderland, 1983, 161-185.

Howard E. B., Britt J. O., Matsumoto G. : Parasitic diseases. *In:* Pathobiology of Marine Mammal Disease, E. B. Howard (ed.). *CRC Press,* Boca Raton, 1983, *1,* 119-233.

Johnston T. H., Mawson P. : Internal parasites of the pigmy sperm whale. *Rec. South Australian Mus.,* 1939, *6,* 263-274.

Johnston T. H., Mawson P. : Nematodes from Australian marine mammals. *Rec. South Australian Mus.,* 1941, *6,* 429-434.

Joyeux C., Baer J. G. : Sur la présence du nématode *Crassicauda crassicauda* (Creplin, 1829) chez un dauphin des côtes de la France. *Bull. Soc. Pathol. Exot.,* 1931, *24,* 198-203.

Lambertsen R. H. : Taxonomy and distribution of a *Crassicauda* species (Nematoda: Spirurida) infecting the kidney of the common fin whale (*Balaenoptera physalus* Linné, 1758). *J. Parasitol.,* 1985, *71* (4), 485-488.

Lambertsen R. H. : Disease of the common fin whale *(Balaenoptera physalus):* Crassicaudosis of the urinary system. *J. Mammal.,* 1986, *67,* 353-366.

Lòpez-Neyra C. R. : Sobre unos vermes paràsitos obtenidos en el *Ziphius cavirostris* Cuvier (Cetaceo; Denticeto) varado en Almeria, en especial de *Crassicauda giliakiana* (Spiruroidea; Tetrameridae). *Rev. Ibèr. Parasitol.,* 1958, *18,* 13-18.

Margolis L., Arai H. P. : Parasites of Marine Mammals. *In:* Synopsis of the Parasites of Vertebrates of Canada, M. J. Kennedy (ed.). *Alberta Agriculture, Animal Health Division,* 1989, 26 p.

Margolis L., Pike G. C. : Some helminth parasites of Canadian Pacific whales. *J. Fis. Res. Board Can.,* 1955, 12, 97-120.

Margolis L., Esch G. W., Holmes J. A., Kuris A. M., Schad G. A. : The use of ecological terms un parasitology (report of an *ad hoc* committee of the American Society of Parasitologists). *J. Parasitol.,* 1982, *68,* 131-133.

Norusis M. J. : Manual of the SPSS for the IBM PC/XT. *SPSS Inc.,* Chicago, 1984, 307 p.

Perrin W. F., Powers J. E. : Role of a nematode in natural mortality of spotted dolphins. *J. Wildl. Manage.,* 1980, *44,* 960-963.

Raga J. A. : Redescription de *Crassicauda grampicola* Johnston et Mawson, 1941 (Nematoda: Spirurida), parasite de *Grampus griseus* (Cuvier, 1812) (Cetacea : Delphinidae). *Vie Milieu,* 1987, *37,* 215-219.

Raga J. A. : Parasitism in Cetacea. *In:* Handbuch der Säugetiere Europas, Meeressäuger. *Aula Verlag,* Wiesbaden (in press).

Raga J. A., Casinos A., Filella S., Raduán M. A. : Notes on Cetaceans of the Iberian coasts. V. *Crassicauda grampicola* Johnston and Mawson, 1941 (Nematoda) cause of injuries in the pterygoids of some specimens of *Grampus griseus. Säugetierk. Mitt.,* 1982, *30,* 315-318.

Robineau D. : Lésions osseuses liées à la présence de vers parasites du genre *Crassicauda* (Nematoda, Spiruroidea) sur la face ventrale d'un crâne de *Tursiops truncatus* (Cetacea, Delphinidae). *Ann. Soc. Sci. Charente-Marit.,* 1975, *6,* 93-97.

Skrjabin A. S. : New helminth species from marine mammals in the Pacific Ocean and Far-east Sea. *Izvestiya Krimskovo Pedagogicheskogo Instituta M. V. Frunze,* 1959, *34,* 99-118 (in Russian).

Skrjabin A. S. : A new *Crassicauda (Crasssicauda delamureana* n. sp.) a parasite of the sei whales. *Craevaya Parazitologiya y prirodnaya ochagovast transmissivnij bolieznii. Seriya Problemy parazitologii,* Kiev, 1966, 100-107 (in Russian).

Skrjabin A. S. : A new nematode, *Crassicauda costata* sp. n., a parasite of the southern whale. *Parazitologiya (Lening.),* 1969, *3,* 258-264 (in Russian).

Skrjabin A. S. : New data in the study of the species *Crassicauda crassicauda* (Nematoda, Spirurida). *Biol. Nauki (Mosc.),* 1973, *16,* 7-12 (in Russian).

Skrjabin K. I., Andreewa N. K. : Un nouveau Nématode : *Crassicauda giliakiana* n. sp. trouvé dans les reins de *Delphinapterus leucas. Ann. Parasitol. Hum. Comp.,* 1934, *12,* 15-28.

Sokal R. R., Rohlf F. J. : Biometry, second edition. *W. H. Freeman and Company,* New York, 1981, 859 p.

Spaul E. A. : *Crassicauda bennetti* sp. n., a new nematode parasite from the bottle-nosed whale *(Hyperoodon). Ann. Mag. Nat. Hist.,* 1926, *17* (9), 581-585.

Ann. Parasitol. Hum. Comp., 1990, *65* : n° 5-6, 262-266.

Mémoire.

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Mots-clés : Tique. *Ixodes ricinus.* Immunité. Lapin. Cyclosporin A. Immunosuppression.

RABBITS INFESTED WITH *IXODES RICINUS* L. ADULTS: EFFECTS OF A TREATMENT WITH CYCLOSPORIN A ON THE BIOLOGY OF TICKS FED ON NAIVE AND IMMUNE HOSTS

P. GIRARDIN, M. BROSSARD

SUMMARY

Rabbits have been infested 3 times with 10 female and 10 male *Ixodes ricinus*. Immunity which is induced when ticks feed on naive animals (1st infestation) perturbs feeding, oviposition and embryogenesis during reinfestations. Treatment of rabbits during a 3rd infestation (resistant animals) with cyclosporin A (CsA), an immunosuppressive agent which works on the cellular compartment (chiefly T helper cells), partially reversed the negative effects of the immunity on the biology of the ticks.

Conversely, CsA may also directly affect the reproductive processes of ticks. Thus, the weight of the eggs laid and the egg conversion factor of ticks fed on naive treated hosts (1st infestation) were diminished. In addition, the preoviposition was prolonged, and finally failure in oviposition and hatching occured more frequently.

RÉSUMÉ : **Infestations de lapins par les adultes d'*Ixodes ricinus* L. : effet d'un traitement par la cyclosporine A sur la biologie des tiques nourries sur des hôtes indemnes ou immuns.**

L'intervention du compartiment cellulaire dans l'immunité de lapins contre les tiques *Ixodes ricinus* a été montrée en traitant les animaux par la cyclosporine A (CsA), un immunosuppresseur agissant essentiellement sur les cellules T auxiliaires.

Des lapins ont été infestés à 3 reprises par 10 femelles et 10 mâles d'*I. ricinus*. L'immunité induite durant la première infestation perturbe la nutrition, l'oviposition et l'embryogenèse des tiques lors de réinfestations. Le traitement des animaux par la CsA au cours d'une 3e infestation inhibe ces effets défavorables.

D'autre part, la CsA affecte directement le processus de reproduction. En effet, les pontes des tiques de première infestation, nourries sur des lapins traités, sont moins abondantes. La préoviposition est aussi prolongée, alors que l'oviposition et l'éclosion des œufs échouent plus fréquemment.

INTRODUCTION

The immune response of rabbits to adult *I. ricinus* bites has been characterized (Bowessidjaou *et al.*, 1977; Brossard, 1977; Brossard *et al.*, 1982; Brossard, Girardin, 1979; Brossard, Fivaz, 1982; Girardin, Brossard, 1985; Girardin, Brossard, 1989). This response leads to a state of resistance against the ticks after repeated infestations (Bowessidjaou *et al.*, 1977; Brossard *et al.*, 1982). Thus, the average duration of the bloodmeal, preoviposition and embryogenesis are prolonged and engorgement, egg laying frequency, egg conversion factor (weight of eggs laid/weight of fed female), as well as hatching frequency are diminished.

Cyclosporin A (CsA), an immunosuppressive agent largely applied in transplantation surgery, inhibits rejection of allografts (Borel, 1981; Britton, Palacios, 1982), as well as graft versus host reactions (Borel *et al.*, 1977; Powles *et al.*, 1980). The properties of this metabolite make it an attractive tool in the investigation of the immune response (Parker *et al.*, 1984; Truffa-Bachi, 1987; Girardin, Brossard, 1989).

Recently we have demonstrated (Girardin, Brossard, 1989), that CsA: (1) diminishes the secondary humoral response of rabbits to female *I. ricinus* bites, (2) blocks the immediate cutaneous reaction (type 1) normally developed by re-infested animals after the intradermal injection of tick salivary gland antigens and (3) impairs the delayed type hypersensitivity reaction (type IV) of the hosts to the same antigen administration.

In the present study, we analysed the effects of treating rabbits with CsA during a 1st or 3rd infestation on the biology of *I. ricinus* ticks. We considered the following parameters: (1) the duration of the bloodmeal; (2): the weight of the engorged females; (3): the duration of preoviposition; (4) the weight of the egg layings; (5): the egg conversion factor; (6): the duration of the embryogenesis; (7): the yield of the parasitic cycle (egg laying and hatching frequencies).

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MATERIAL AND METHODS

Host animals

Male Himalayan rabbits of genotype aac^Hc^H were infested 1 or 3 times with 10 male and 10 female *I. ricinus*. Only females of this species engorge, but copulation is necessary for an optimal bloodmeal (Graf, 1978a). A 15 day delay separated the infestation periods. For each infestation, 5 rabbits were treated with CsA. During the 1st infestation, 6 rabbits served as controls and 5 control animals were used for the 3rd infestation. The hosts were aged from 3 months (1st infestation) to 5 months (3rd infestation) and weighed 2.5 kg on average.

Ticks

The *I. ricinus* ticks used in our experiments were reared in our laboratory according to the method of Graf (1978b). During the infestations, the ectoparasites were confined within a cloth bag placed over the ear of the rabbit. A collar prevented the host from grooming. Engorged ticks were taken, weighed and reared.

IMMUNOSUPPRESSIVE TREATMENT

As described by Lindsey *et al.* (1980), we dissolved the CsA (Sandoz AG, Basel) in mineral oil (Miglyol 812, dynamit nobel). A daily dose of 25 mg/kg was administrated to the rabbits subcutaneously. Control animals were subjected to subcutaneous injections of the single solvent (0.5 ml mineral oil per day). Considering the strength of the immune response (Papatheodorou, 1985; Girardin, 1987) and that the resistance extends the period of tick feeding (Bowessidjaou *et al.*, 1977), we performed the treatment on days -1 to $+6$ for the 1st infestation and on days -2 to $+8$ with the 3rd infestation.

Statistics

We used the non parametric Mann-Withney test (Siegel, 1956) for the comparison of the data obtained for each variable, except for the yield of the parasitic cycle. In this case, we analysed our results by means of the frequency exact test (Ostle, 1963).

RESULTS

Results are summarized in *Table I*. For each variable, we did « vertical » comparisons of our data (1st infestation versus 3 rd infestation), in order to demonstrate the effects of rabbits resistance on the biology of the ticks, and to determine the influence of CsA on host immunity. A direct effect of CsA on the biology of the ticks was estimated by proceeding to « horizontal » assessment of our results (control versus treated).

1 — *Duration of the bloodmeal*

During the 3rd infestation, the *I. ricinus* females remained attached to control hosts for a longer time than during the 1st ($p < 0.01$). Treatment of the rabbits diminished the duration of the bloodmeal during the 3rd infestation ($p < 0.001$), but increased it during the 1st ($p < 0.01$).

TABLEAU 1. — *Influence of treating rabbits with CsA during a 1st or a 3rd infestation on the biology of* I. ricinus. *St-dev: standard deviation. For* p *values, see text and figures 1, 2 and 3.*

	INFESTATION 1					
	CONTROLS			TREATED		
	Mean	St-dev	n	Mean	St-dev	n
Duration of the bloodmeal (hours)	163.72	18.47	46	177.93	19.90	45
Weight of the engorged ticks (mg)	226.62	102.76	46	240.66	54.07	45
Duration of the preoviposition (days)	9.67	2.00	36	12.77	8.87	39
Weight of the egg layings (mg)	115.28	50.40	36	83.82	29.96	39
Egg conversion factor	0.43	0.11	36	0.34	0.10	39
Duration of the embryogenesis (days)	36.09	7.59	35	48.45	13.69	31
Egg laying frequency	36/46			39/45		
Hatching frequency	35/36			31/39		

	INFESTATION 3					
	CONTROLS			TREATED		
	Mean	St-dev	n	Mean	St-dev	n
Duration of the bloodmeal (hours)	200.86	26.58	42	179.81	18.25	42
Weight of the engorged ticks (mg)	188.88	88.89	42	201.13	75.93	42
Duration of the preoviposition (days)	16.07	7.11	30	9.46	1.73	28
Weight of the egg layings (mg)	55.92	50.36	30	80.83	33.30	28
Egg conversion factor	0.31	0.16	30	0.34	0.11	28
Duration of the embryogenesis (days)	64.84	8.86	19	45.78	7.50	23
Egg laying frequency	30/42			28/42		
Hatching frequency	19/30			23/28		

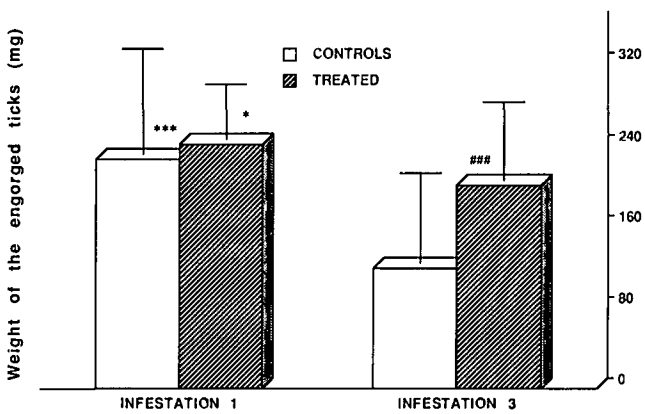

FIG. 1. — Influence of treating rabbits with CsA during a 1st or a 3rd infestation on the engorgement of *I. ricinus* females. Differences between the infestations: * $p < 0.05$, *** $p < 0.001$. Difference between ticks on control or treated hosts: ////// $p < 0.001$.

2 — *Weight of the ticks*

Immune-mediated resistance to tick feeding caused a dramatic decrease in the weight of the engorged ticks *(fig. 1)*. In fact ticks fed both on control and treated rabbits took less blood during the 3rd infestation ($p < 0.001$ for « control » ticks, $p < 0.05$ for ectoparasites fed on treated hosts).

However, the feeding of ticks on multi-infested rabbits was favored by the treatment ($p < 0.001$). During the 1st infestation, the engorgement was only slightly higher in the females on treated hosts.

3 — *Duration of the preoviposition*

Ticks fed on control rabbits laid their first eggs later following the 3rd infestation than after the 1st ($p < 0.001$).

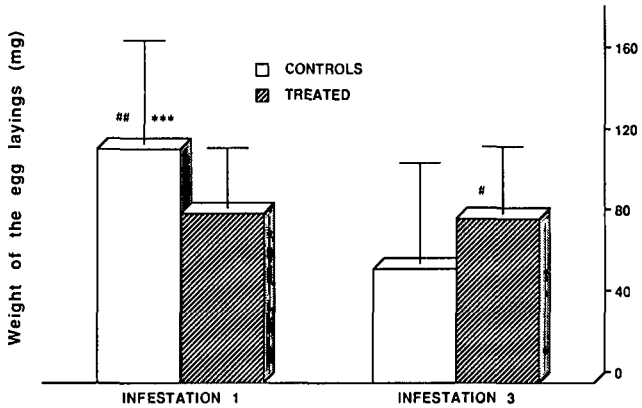

FIG. 2. — Influence of treating rabbits with CsA during a 1st or a 3rd infestation on the weight of egg layings of *I. ricinus* females. Difference between the infestations: *** $p < 0.001$. Differences between ticks on control or treated hosts: - $p < 0.05$, ////// $p < 0.01$.

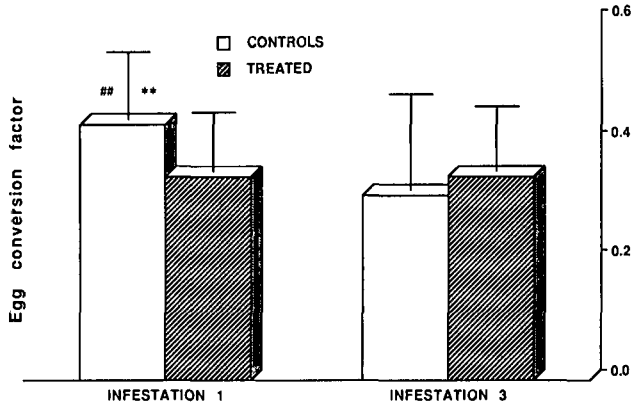

FIG. 3. — Influence of treating rabbits with CsA during a 1st or a 3rd infestation on the egg conversion factor of *I. ricinus* females. Difference between the infestations: ** $p < 0.01$. Difference between ticks on control or treated hosts: ////// $p < 0.01$.

However when engorged on treated hosts an opposite effect was observed ($p < 0.01$). CsA clearly impaired expression of the resistance on the preoviposition. In fact, ticks fed on treated rabbits laid their eggs earlier than did those fed on control animals after a 3rd infestation ($p < 0.001$). However, the drug may have directly disturbed the oogenesis, because treatment delayed egg deposition during the 1st infestation ($p < 0.05$).

4 — *Weight of the egg layings*

Immune-mediated resistance caused a decrease in the weight of the egg layings from females fed on control rabbits ($p < 0.001$, *fig. 2*). This adverse influence was suppressed by administration of CsA to hosts, since no difference was detected between the egg layings of ticks engorged on treated animals.

We observed opposite effects of CsA treatment during a 1st or a 3rd infestation. The egg layings from females fed on treated hosts were smaller after a 1st infestation ($p < 0.01$) and larger after a 3rd infestation ($p < 0.05$) than those from ticks on control animals, suggesting that CsA may exert an adverse direct action on the mechanisms of egg production.

5 — *Egg conversion factor*

With the control ticks, the valucs recorded in the present study for the egg conversion factor were less than those obtained by Graf (1978a) or Papatheodorou (1985). Nevertheless, this variable decreased dramatically in the course of successive infestations ($p < 0.01$, *fig. 3*). This difference was abolished by CsA treatment.

The egg conversion factor for females engorged on rabbits treated during a 1st infestation was greatly diminished when compared with that obtained for ticks fed on control hosts ($p < 0.01$). This again confirms the harmful action of CsA on the reproduction mechanisms of the ectoparasites.

6. *Duration of the embryogenesis*

Embryogenesis of eggs laid by females fed on control rabbits was quicker after a 1st than after a 3rd infestation ($p < 0.001$). The ectoparasites engorged on treated animals during the 1st or the 3rd infestation laid eggs which hatched within the same delay.

After a 3rd infestation, eggs of ticks fed on treated hosts hatched earlier than if engorged on control animals ($p < 0.01$). For the 1st infestation, we observed the contrary ($p < 0.05$). Thus the drug may also directly disturb embryogenesis.

7 — *Egg laying and hatching frequencies*

The resistance acquired by control rabbits only slightly affected the egg laying frequency. On the other hand, we observed a significant decrease of this proportion after a 3rd infestation in the ticks of the treated group ($p < 0.05$). Host immunity to *I. ricinus* had a dramatic action on the hatching frequency: this ratio was lower in control tick of 3rd infestation than those of 1st infestation ($p < 0.001$). The effect was abolished under the influence of the immunosuppressive treatment.

Eggs issued from females after a 1st infestation on treated rabbits hatched in a significantly lower proportion than those from the control population ($p < 0.05$). This was not the case after a 3rd infestation.

DISCUSSION

Previous studies demonstrate that the immune response directed against female *I. ricinus* disrupts many of the feed-

ing and digestive (Girardin, 1987; Brossard, Papatheodorou, 1990) as well as reproductive (Bowessidjaou *et al.*, 1977; Brossard *et al.*, 1982) mechanisms of these ticks. The resistance phenomenon is obviously detectable during a re-infestation, and markedly amplified during a 3rd infestation. Our results obtained in ticks fed on control rabbits confirm these observations.

Different immunosuppressive treatments allow an attenuation or a blockade of the resistance developed by hosts against ticks. Thus Allen (1973), and then Wikel and Allen (1976) have obtained these effects on guinea pigs resistant to *Dermacentor andersoni* larvae, using the cytostatic drugs methotrexate and cyclophosphamide, respectively.

In the present study, we treated rabbits with CsA during a 1st or a 3rd infestation with female *I. ricinus*. This regimen attenuated the effects of the resistance of the hosts against the ectoparasites during the 3rd infestation. Consequently the feeding and preoviposition periods were then reduced, while the bloodmeal and the egg layings were generally more voluminous. Moreover eggs laid by females engorged on hosts treated during a 3rd infestation hatched earlier.

We postulated that the favorable effect of the treatment for the ticks is due essentially to the depression of the immune system of the rabbit by the drug. In a previous study we demonstrated with our system the influence of CsA on the secondary antibody response, as well as on the cutaneous immediate and delayed type hypersensitivity reactions to tick salivary antigens (Girardin, Brossard, 1989). This was roughly a consequence of the effects of the drug on the cellular component of the immune system (chiefly the T helper cells) described in detail by others (Borel *et al.*, 1977; Borel, Lafferty, 1983; Thomson *et al.*, 1983a; Thomson *et al.*, 1983b). In another system CsA may also cause perturbations of the development of *Schistosoma mansoni* through its effects on the immune system of the host (Bueding *et al.*, 1981; Nilsson *et al.*, 1985; Thomson *et al.*, 1986).

However, as we observed, the indirect beneficial effects for ticks provided by the immunosuppressive agent, were partially counterbalanced by an injurious direct action on the tick reproductive success. The later effect was obviously observable during the 1st infestation. Thus, the detachment of ticks from treated rabbits was delayed, leading to a prolonged and greater exposition to the drug, and to the components of the growing host's immune response. Females, when engorged on treated hosts, layed lighter and late egg layings. Furthermore, the average egg conversion factor for females fed on treated rabbits, on the one hand was lower than that recorded for control ticks, on the other hand, was equivalent to that normally observed by ticks engorged on resistant hosts. In addition, CsA caused a dramatic failures in the hatching of eggs. This drug may also exert a direct adverse action against various Plasmodia (Nickell *et al.*, 1982), rodent filaria and trichina (Borel, Lafferty, 1983).

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RÉFÉRENCES

Allen J. R. : Tick resistance: basophils in skin reactions of resistant guinea pigs. *Int. J. Parasitol.*, 1973, *3*, 195-200.

Borel J.-F., Feurer C., Magnee L., Staehelin H. : Effects of the new anti-lymphocytic peptide cyclosporin A in animals. *Immunology*, 1977, *32*, 1017-1025.

Borel J.-F. : Cyclosporin-A: present experimental status. *Transplant. Proc.*, 1981, *13*, 344-348.

Borel J.-F., Lafferty J. : Cyclosporin: speculation about its mechanism of action. *Transplant. Proc.*, 1983, *15*, 1881-1885.

Bowessidjaou J., Brossard M., Aeschlimann A. : Effects and duration of resistance acquired by rabbits on feeding and egg laying in *Ixodes ricinus* L. *Experientia*, 1977, *33*, 548-550.

Britton S., Palacios R. : Cyclosporin A. — Usefulness, risks and mechanism of action. *Immunol. Rev.*, 1982, *65*, 5-22.

Brossard M. : Rabbits infested with the adults of *Ixodes ricinus* L.: Passive transfer of resistance with immune serum. *Bull. Soc. Pathol. Exo.*, 1977, *70*, 289-294.

Brossard M., Fivaz V. : *Ixodes ricinus* L.: mast cells, basophils and eosinophils in the sequence of cellular events in the skin of infested or reinfested rabbits. *Parasitology*, 1982, *85*, 583-592.

Brossard M., Girardin P. : Passive transfer of resistance in rabbits infested with adult *Ixodes ricinus* L.: Humoral factors influence feeding and egg laying. *Experientia*, 1979, *35*, 1395-1396.

Brossard M., Monneron J. P., Papatheodorou V. : Progressive sensitization of circulating basophils against *Ixodes ricinus* L. antigens during repeated infestations of rabbits. *Parasite Immunol.*, 1982, *4*, 355-361.

Brossard M., Papatheodorou V. : Immunity against female *Ixodes ricinus* L.: effect on feeding and haemoglobin digestion. *Ann. Parasitol. Hum. Comp.*, 1990, *65*, 32-36.

Bueding E., Hawkins J., Cha Y.-N. : Antischistosomial effects of cyclosporin A. *Agent Actions*, 1981, *11*, 380-393.

Girardin P. : Immunité du lapin contre la tique *Ixodes ricinus* L. (Ixodoidea, Ixodidae): mécanismes effecteurs et leurs effets sur la biologie de l'ectoparasite. *Ph. D. Thesis*, 1987, Neuchâtel.

Girardin P., Brossard M. : Développement d'une hypersensibilité retardée chez des lapins infestés par les femelles d'*Ixodes ricinus* L. *Ann. Parasitol. Hum. Comp.*, 1985, *60*, 299-309.

Girardin P., Brossard M. : Effects of cyclosporin-A on the humoral immunity to ticks, and on the cutaneous immediate (type I) and delayed (type IV) hypersensitivity reactions to *Ixodes ricinus* L. salivary gland antigens in re-infested rabbits. *Parasitol. Res.*, 1989, *75*, 657-662.

Graf J.-F. : Copulation, nutrition et ponte chez *Ixodes ricinus* L. (Ixodoidea: Ixodidae). 1re partie. *Bull. Soc. Entomol. Suisse*, 1978a, *51*, 89-97.

Graf J.-F. : Copulation, nutrition et ponte chez *Ixodes ricinus* L. (Ixodoidea: Ixodidae). 3e partie. *Bull. Soc. Entomol. Suisse*, 1978b, *51*, 343-360.

Lindsey N. J., Harris K. R., Norman H. B., Smith J. L., Lee H. A., Slapek M. : The effect of cyclosporin A on the primary and secondary immune responses in the rabbit. *Transplant. Proc.*, 1980, *12*, 252-255.

Nickell S. P., Scheibel L. W., Cole G. A. : Inhibition by cyclosporin A of rodent malaria in vivo and human malaria in vitro. *Infect. Immun.*, 1982, *37*, 1093-1110.

Nilsson L. A., Lindblad R., Olling S., Ouchterlony O. : The effect of cyclosporin A on the course of murine infection by *Schistosoma mansoni*. *Parasite Immunol.*, 1985, *7*, 19-27.

Ostle B. : Statistics in research: basic concepts and techniques for research workers: The Iowa state University Press, 1963, p. 132-133.

Papatheodorou V. : Réaction immunitaire du lapin contre la tique *Ixodes ricinus* L. (Ixodoidea: Ixodidae) : effets sur la composition et la digestion du repas sanguin. *Ph. D. Thesis*, 1985, Neuchâtel.

Parker D., Droessler K., Turk J. L. : Kinetics of a single dose of cyclosporin-A on antibody and cell mediated immune response in the guinea pig. *Int. J. Immunopharmacol.*, 1984, *6*, 67-74.

Powles R. C., Clink H. M., Hedley D., Bandini G., Watson J. G., Lawson D., McElwain T. J., Alexander P. : Cyclosporin A to prevent graft-versus-host disease in man after allogeneic bone-marrow transplantation. *Lancet*, 1980, *i*, 327-329.

Siegel S. The Mann-Whitney test. *In:* Non parametric statistics for the behavorial sciences. *McGraw-Hill Book Company Inc., Kogagusha Company Ltd*, Tokyo, 1956, p. 117-127.

Thomson A. W., Moon D. K., Geczy C. L., Nelson D. S. : Cyclosporin A inhibits lymphokine production but not response of macrophages to lymphokines. *Immunology*, 1983a, *48*, 291-299.

Thomson A. W., Moon D. K., Geczy A. F., Nelson D. S. : Suppression of delayed-type hypersensitivity reactions and lymphokine production by cyclosporin A in the mouse. *Exp. Immunol.*, 1983b, *52*, 599-606.

Thomson A. W., Smith S. W. G., Chappell L. H. : Cyclosporin A: Immune suppressant and anti-parasitic agent. *Parasitol. Today*, 1986, *2*, 288-290.

Truffa-Bachi P. : Cyclosporin A: a tool for dissecting the mechanisms of the immune response. *Ann. Inst. Pasteur/Immunol.*, 1987, *138*, 644-648.

Wikel S. K., Allen J. R. : Acquired resistance to ticks. II. Effects of cyclosphosphamide on resistance. *Immunology*, 1976, *30*, 479-484.

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