Studies on the Properdin System in Rats Bearing the Transplantable Human Carcinoma HR132

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In an attempt to explain the natural resistance of animals to heterologously transplanted tumors, it was theorized that the protective mechanism may be due to the properdin system (1, 3). To test this hypothesis it was chosen to study the effect of zymosan on the transplantable human carcinoma of the colon (HR132) carried in weanling Wistar rats (2).

Zymosan was selected rather than properdin, because properdin was unobtainable. It may be recalled that zymosan is an insoluble carbohydrate complex derived from yeast cell walls, that has the property of combining with properdin *in vivo* and thus influencing the properdin level of the blood (6). By actual determination, Pillemer and Ross (5) had previously shown that intravenous injections of small doses (5 and 25 mg/kg) of zymosan produced a drop in properdin titer followed, in 2–14 days, by a rise to as high as 300 per cent above normal. Intravenous injections of larger doses (125 mg/kg) produced even a greater initial fall in properdin titer with a return to only 75 per cent of normal in 6–10 days. Using these findings as a basis, we succeeded in conditioning normally resistant weanling Wistar rats to HR132 by first injecting the animals with varying amounts of zymosan (2). In the experiment, out of a total of 160 animals in each category the following tumor "takes" were obtained: nineteen in nontreated rats, 136 in rats conditioned by 300 r total-body irradiation, 58 in rats given a single injection of zymosan, and 90 in rats given multiple injections of zymosan.

From the data presented it was concluded that zymosan is effective in increasing the susceptibility of weanling Wistar rats to HR132. That this conditioning is mediated through the properdin system, however, was not established, since properdin levels on sera from the animals used were not ascertained. To implicate or rule out the properdin system, it was thus deemed necessary (*a*) to repeat the experiment, with actual determination of properdin titers on sera from the animals at periodic intervals, and (*b*) to see if the resistance of weanling Wistar rats made susceptible to HR-132 could be restored by the administration of properdin.

MATERIALS AND METHODS

The materials and methods used in the experiment were a duplication of those previously employed (2). Briefly, the animals were weanling female Wistar rats weighing between 40 and 50 gm. The tumor was the transplantable human carcinoma of the colon HR132 propagated in our laboratory since 1954 in Wistar rats conditioned by total-body irradiation. Fleischmann's zymosan (Lot #5B-171) was prepared for use by the method of Pillemer *et al.* (4). Properdin, derived from human blood, was prepared and supplied by Pillemer, and properdin titers on animal sera were determined by Pillemer and Todd.

Actually, three separate experiments were performed. Experiments I and II duplicated the experiment previously recorded (2), with the exception that properdin titers were determined on sera from the animals used. Experiment III was designed to study the effect of administration of properdin on the growth of HR132 in weanling Wistar rats made susceptible to the implanted tumor by exposure to 300 r total-body irradiation.

More specifically, Experiment II was carried out as a check on Experiment I and, by using a greater number of rats, was planned to compare the number of tumor "takes" or regressions with those obtained in the series previously reported (2). In each of *Experiments I and II* the animals were divided into two groups—one not subjected to tumor implantation and the other inoculated subcutaneously with fragments of a 10-day-old donor tumor on the 4th day of the experiment. The animals in each main group were subdivided into the following five subgroups, depending upon the treatment received: (*a*) none, (*b*) 300 r total-body irradiation given on the 3d day of the experiment, (*c*) a single injection of 15 mg of zymosan/kg of body weight given on the 1st day of the experiment, (*d*) a single injection of 125 mg of zymosan/kg given on the 1st day

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of the experiment, and (*e*) multiple injections of 125 mg of zymosan/kg, given on the 4th, 9th, 11th, and 16th days of the experiment. All zymosan was administered intravenously by way of tail veins.

Serum for determination of properdin titers was collected on the 2d day of the experiment from animals (*a*) receiving no treatment, (*b*) treated with a single injection of 15 mg of zymosan/kg, and (*c*) given a single injection of 125 mg of zymosan/kg. Thereafter, it was collected from animals in each group (except nontreated without tumors, which were taken sporadically, since these were normal animals) on the following days of the experiment: 4th, 6th, 8th, 10th, 12th, 15th, 17th, and 18th. Two animals were sacrificed in obtaining the serum for each properdin determination in Experiment I, while only one was sacrificed for each determination in Experiment II.

In *Experiment III* all animals were implanted with HR132 and were divided into the following four groups, depending upon the treatment received: (*a*) none; (*b*) 300 r total-body irradiation given 24 hours before implantation of tumor; (*c*) 200 units properdin followed in 2 hours by 300 r total-body irradiation, in 2 additional hours by 200 units properdin, the following four mornings by 200 units properdin, and each morning and evening thereafter by 200 units properdin, for a total of 25 injections; and (*d*) 300 r total-body irradiation, followed in 2 hours and each morning thereafter by 200 units properdin until the experiment was terminated, for a total of fifteen injections. All properdin was given by way of tail veins. Serum was collected for properdin determination from one animal in each group (including a normal control) and sacrificed on the following days of the experiment: 2, 4, 6, 9, 13, and 15. In animals treated with properdin, collection of blood was carried out 4 hours after the last injection.

Animals were weighed at regular intervals throughout the experiments. Tumors were measured (in mm.) in three dimensions, and the average of these was taken as the diameter. All surviving animals were sacrificed on the 18th day of Experiments I and II (equivalent to the 14th day of implantation of the tumor in the second main group) and on the 15th day of Experiment III (also equivalent to the 14th day of implantation of tumor). A complete autopsy was performed on the animals which were sacrificed periodically to obtain serum for properdin titers as well as on those sacrificed at the termination of the experiment. Microscopic examination was made of each tumor or tumor site and also of the liver, kidneys, spleen, lymph nodes, and thymus from each animal.

RESULTS AND DISCUSSION

The results obtained in Experiment I are tabulated in Table 1. The first portion of the table discloses the type of treatment, the days on which zymosan was injected, and the properdin titers of sera from rats not implanted with tumors. It will be noted that the properdin levels (units/ml) (*a*) were 18 in normal rats, (*b*) varied between 18 and 6 in rats exposed to 300 r, (*c*) first declined to 12 and then rose to as high as 36 in rats given a single injection of 15 mg of zymosan/kg of body weight, (*d*) first declined to 6 and then rose to as high as 48 in rats given a single injection of 125 mg of zymosan/kg, and (*e*) declined to 3 and remained at 9 and 12 except for a level of 18 on the last day of the experiment, in rats given four injections of 125 mg of zymosan/kg.

The second portion of Table 1 reveals the re-

TABLE 1

EFFECT OF ZYMOSAN (Z) ON PROPERDIN TITERS (UNITS/ML) OF SERA FROM RATS IN EXPERIMENTS I AND II

Exp.	Group	Treatment	Day of treatment 1	2	4	6	8	9	10	11	12	15	16	17	18
I	No tumors	None		18											
		300 r			18	9	9		9		6	12		18	
		15 mg zymosan/kg×1	Z	12	24	36	24		24		24	24		18	18
		125 mg zymosan/kg×1	Z	6	24	24	36		48		36	36		24	24
		125 mg zymosan/kg×4			Z3*	12	12	Z	12	Z	12	9	Z	12	18
	Tumors	None				18	24		24		24R	24R		24R	24R
		300 r			24	12	6		3		6G	6G	Z	6G	6G
		15 mg zymosan/kg×1	Z	9	12	24	24		24		36R	24R		24R	18R
		125 mg zymosan/kg×1	Z	9	9	9	12		12		18G	12G		36R	36R
		125 mg zymosan/kg×4			Z3*	9	12	Z	9	Z	6G	24G	Z	24R	36R
II	No tumors	None		24					24		24	24		18	24
		300 r			18	18	18		18		9	6		12	12
		15 mg zymosan/kg×1	Z	12	12	24	36		24		18	18		18	18
		125 mg zymosan/kg×1	Z	6	9	18	24		24		24	18		18	18
		125 mg zymosan/kg×4			Z4*	6	24	Z	18	Z	12	30	Z	18	9
	Tumors	None				18	18		18		24R	24R		36R	18R
		300 r			18	24	24		9		24G	6G		9G	9G
		15 mg zymosan/kg×1	Z	30	24	24	18		24		24R	24R		30R	24R
		125 mg zymosan/kg×1	Z	6	30	24	24		24		24R	24R		36G	24G
		125 mg zymosan/kg×4			Z18*	18	18	Z	6	Z	9G	6G	Z	24G	18G

* Properdin determined 4 hours after injection of zymosan.
×1 indicates a single injection of zymosan. ×4 indicates four injections of zymosan.
R indicates regression of tumor.
G indicates growth of tumor.

sults obtained in rats similarly treated but, in addition, implanted with HR132. Since the experiment was designed primarily for the obtaining of properdin titers on animal sera the number of rats used was, unfortunately, insufficient to establish anything about the incidence of tumor "takes" or regressions. Ordinarily, even under optimum conditions, such calculations cannot be made before the 8th day after implantation of HR132, because it is impossible to determine whether the tumor will or will not "take" before this date, owing to its relatively slow rate of growth. In this experiment, most of the animals were already sacrificed by the 8th day after the tumor was inoculated. From the table it will be noted that the properdin titers (*a*) rose to 24 in rats receiving no treatment and showing regression of tumor; (*b*) declined to a low of 6 in rats exposed to 300 r and revealing growth of tumor; (*c*) rose to a high of 36 in rats given a single injection of 15 mg of zymosan/kg and disclosing regression of tumor; (*d*) declined to a low of 12 in rats given a single injection of 125 mg of zymosan/kg and showing growth of tumor, but rose to a high of 36 in similarly treated rats but revealing regression of tumor; and (*e*) declined to a low of 6 in rats given 4 injections of 125 mg of zymosan/kg and disclosing growth of tumor, but rose to a high of 36 in similarly treated rats showing regression of tumor. In this category, however, there was also a properdin level of 24 in a rat that disclosed growth of tumor. This animal was sacrificed on the 15th day of the experiment —11 days after the tumor had been implanted and established. These results thus seem to negate any definite and consistent relationship of properdin levels in Wistar rats with their resistance or susceptibility to HR132.

To check these data and to see whether the incidence of tumor "takes" reported previously (2) could be sustained, the entire procedure was duplicated in Experiment II with a slightly larger number of animals. The results are recorded in Table 1. In tumor-free animals the properdin levels (*a*) were 18 and 24 (with the majority 24) in normal rats, (*b*) varied between 6 and 18 (with the majority 12 and 18) in rats exposed to 300 r, (*c*) first declined to 12 and then rose to a high of 36 in rats given a single injection of 15 mg of zymosan/kg of body weight, (*d*) first declined to 6 and then rose to as high as 24 in rats given a single injection of 125 mg of zymosan/kg, and (*e*) declined to 4 but rose to a high of 30 in rats given four injections of 125 mg of zymosan/kg.

In tumor-bearing animals the data, as noted in Table 1, disclose that properdin titers (*a*) varied between 18 and 36 in animals receiving no treatment and showing regression of tumor, (*b*) varied between 9 and 24 in rats exposed to 300 r and disclosing growth of tumor, (*c*) showed an initial elevation and remained at 24 and 30 in rats given a single injection of 15 mg of zymosan/kg and revealing regression of tumor, (*d*) disclosed a fall to 6 and remained at 24 and 36 in rats showing growth of tumor and 24 in a rat showing regression of tumor in animals given a single injection of 125 mg of zymosan/kg, and (*e*) varied between 6 and 24 in animals given four injections of 125 mg of zymosan/kg and disclosing growth of tumor. In other words, the properdin levels varied between 24 and 36 in each of the animals in which the tumor regressed except one in which the level was 18. On the other hand, they varied between 6 and 36 in each of the animals in which the tumor grew. Thus, the data in Experiment II indicate complete lack of correlation between properdin titers and resistance or susceptibility to HR132.

The number and size of tumor "takes" in Experiment II are indicated in Table 2. Based on a survival of 8 days or longer, results indicate (*a*) that there were no growths in eleven untreated rats, (*b*) that there were fourteen growths in sixteen rats subjected to 300 r total-body irradiation, (*c*) that there were two growths in thirteen rats given a single injection of 15 mg of zymosan/kg, (*d*) that there were four growths in thirteen rats given a single injection of 125 mg of zymosan/kg, and (*e*) that there were seven growths in fourteen rats given four injections of 125 mg of zymosan/kg. While not as good, these results are comparable to those previously recorded (2). They do indicate that weanling Wistar rats can be conditioned to HR132 by zymosan but that such conditioning is not as effective as that obtained by exposure to 300 r total-body irradiation.

Finally, as far as Experiments I and II are concerned, it should be noted that the pathologic changes in organs of the reticuloendothelial system were similar to those previously observed (2). Briefly, the liver, spleen, lymph nodes, and thymus (*a*) from untreated animals and from those receiving 15 mg zymosan/kg × 1 showed no alterations, (*b*) from animals exposed to 300 r total-body irradiation disclosed reticuloendothelial atrophy, and (*c*) from animals treated with 125 mg zymosan/kg × 1 and × 4 revealed reticuloendothelial hyperplasia. In addition, the livers from three tumor-bearing rats treated with 300 r in Experiment II showed focal abscesses. The kidneys from animals in each of the subgroups exhibited no noteworthy changes.

In Experiment III exposure to 300 r total-body irradiation rather than treatment with zymosan

was chosen in conditioning rats for implantation of HR132 prior to administration of properdin because (*a*) exposure to irradiation (as demonstrated in Experiment I, following which the decision was made) produced a more uniform depression of properdin levels, (*b*) the number of tumor "takes" was consistently higher after such treatment, and (*c*) the danger of administering and storing excessive amounts of material (zymosan) within the animal body which might later neutralize the properdin administered was obviated.

The results of Experiment III are shown in Table 2. As noted, the data indicate (*a*) that there were eleven animals in each group that survived 8 days or longer, at which time it could be determined whether the tumors were or were not growing, (*b*) that there were five growths in nontreated animals with the average diameter of the tumors being 7 mm., (*c*) that there were eleven growths in animals exposed to 300 r total-body irradiation with the average diameter of the tumors being 12 mm., (*d*) that there were ten growths in animals given properdin, 300 r, and properdin × 25, with the average diameter of the tumors being 9 mm., (*e*) that there were eleven growths in animals given 300 r and properdin × 15 with the average diameter of the tumors being 11 mm., and (*f*) that the average weights of the irradiated animals were approximately the same regardless of whether they did or did not receive properdin. As far as the death of animals and focal necrosis of the liver, respectively, were concerned, the results were as follows: (*a*) nontreated—1 and 0, (*b*) 300 r—1 and 2, (*c*) properdin, 300 r, and properdin × 25—4 and 4, and (*d*) 300 r and properdin × 15—4 and 1. In other words, administration of properdin did not protect the animals against irradiation. Pathologically, the lymphoid structures showed marked atrophy in animals exposed to 300 r, while they were only slightly, if at all, better preserved in animals receiving properdin in addition.

As indicated in Table 3, the properdin titers of sera from animals in Experiment III (*a*) were consistently at 18 in normal tumor-free animals, (*b*) varied between 12 and 24 in nontreated tumor-bearing animals, with levels at 12 and 24 in two animals in which the tumors grew and at 24 in one animal in which the tumor regressed, (*c*) were 12

TABLE 2

NUMBER AND SIZE (MM.) OF "TAKES" OF HR132 AND WEIGHTS OF RATS IN EXPERIMENTS II AND III

Exp.	Treatment	No. rats used	No. rats surviving*	No. growths*	Av. diameter tumors (mm.)	Wt. of animals Initial (gm.)	Wt. of animals Terminal (gm.)
II	None	14	11	0	0	42	90
	300 r	20	16	14	13	47	67
	15 mg zymosan/kg×1†	18	13	2	9	44	95
	125 mg zymosan/kg×1	18	13	4	8	45	91
	125 mg zymosan/kg×4	18	14	7	9	42	74
III	None	15	11	5	7	51	83
	300 r	15	11	11	12	52	67
	Properdin, 300 r, properdin×25‡	15	11	10	9	48	62
	300 r, properdin×15‡	15	11	11	11	49	59

* Includes those rats that survived for more than 8 days.

† ×1 indicates one injection of zymosan: ×4 indicates four injections of zymosan.

‡ See "Materials and Methods" for details of treatment.

TABLE 3

PROPERDIN TITERS (UNITS/ML) OF SERA FROM RATS IN EXPERIMENT III

Treatment	Day of treatment 2	4	6	9	13	15
Normal (nontumor)	18	18	18	18	18	18
None	24	18	18	12G	24	24R
						24G
300 r	12	18	18G	12G	18G	18G
Properdin, 300 r, properdin×25*			36G	36G	48G	24R
						18G
300 r, properdin×15*	36	36	24G	24G	24G	48G

* See "Materials and Methods" for details of treatment.
R indicates regression of tumor.
G indicates growth of tumor.

and 18 in tumor-bearing animals exposed to 300 r total-body irradiation with a level of 12 in one and levels of 18 in three animals in which the tumors grew, (*d*) varied between 18 and 48 in tumor-bearing animals treated with properdin, 300 r, and properdin × 25, with levels of 18, 36, and 48 in four animals in which the tumors grew and a level of 24 in one animal in which the tumor regressed, and (*e*) varied between 24 and 48 in tumor-bearing animals treated with 300 r and properdin × 15 with levels at 24 in three animals and a level at 48 in one animal in which the tumors grew. In other words, the properdin levels varied between 12 and 48, with all but two below the normal of 18 in animals in which the tumors grew, and they were 24 in two animals in which the tumors regressed.

The data garnered from each of the experiments herein recorded indicate treatment of weanling Wistar rats with zymosan did effectively condition them to the transplantable human carcinoma of the colon HR132. That this conditioning is mediated through alteration of host characteristics other than those reflected simply in the properdin levels of their sera is indicated by the fact that the properdin titers of the sera from such animals as well as from those exposed to 300 r total-body irradiation were slightly depressed, normal, or elevated. It is further evident that the administration of *human* properdin to irradiated weanling Wistar rats increased the properdin titers of their sera to almost 3 times normal and yet did not restore their resistance to implants of the human carcinoma HR132. For these reasons, it is concluded (*a*) that the innate resistance of weanling Wistar rats to the transplantable human carcinoma of the colon HR132 is not mediated through the properdin system, (*b*) that properdin was administered at the wrong time, (*c*) that pretreatment with roentgen rays alters other critical factors, rendering the administered properdin ineffective, or (*d*) that heterologous properdin is ineffective, even though it can be detected in the animals by zymosan assay.

SUMMARY

The hypothesis that the properdin system may play a role in the natural resistance of animals to transplantable tumors was investigated by the use of the transplantable carcinoma of the human colon HR132 in normally nonsusceptible weanling Wistar rats.

In a duplication of a previous experiment, it was again shown that the rats can be conditioned to the human tumor by intravenous injection of appropriate doses of zymosan but that such conditioning is not as effective as that produced by exposure to 300 r total-body irradiation.

The properdin levels of sera from animals so conditioned showed no constant relationship to tumor "takes" inasmuch as some were slightly depressed, others were normal, and others still were elevated.

The administration of *human* properdin to irradiated animals increased the properdin titers of their sera to almost threefold, yet it did not restore their resistance to implants of the human tumor.

It is thus concluded that, under the conditions of the experiments, the natural resistance of weanling Wistar rats to the transplantable carcinoma of the human colon HR132 is not mediated through the properdin system.

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