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Serum Properdin Levels and Cancer Cell Homografts in Man.* (23550)

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Homotransplantation studies with cultivated human cancer cells revealed differences in the ability of healthy persons and patients with advanced cancer to reject the implanted cells(1). The present paper reports parallel differences in serum properdin(2) levels in these same persons. The implantation of cancer cells into normal volunteers elicited a marked local inflammatory reaction and rapid rejection of the implant. In patients with advanced cancer the initial inflammatory reaction was minimal and growth of the implanted cells occurred at almost all sites. Spontaneous regression occurred in some cancer patients but in others growth of the implanted cells was progressive. Attempts have been made to determine the cause of the difference of response of healthy persons and cancer patients to cancer cell implants. None of these studies, except the present investigation, has thus far revealed any gross alterations in cellular or humoral factors that might explain the impairment of homotransplant rejection in the cancer patients (see Discussion). It has been reported elsewhere that treatment of mice and other experimental animals and man with large doses of zymosan or certain polysaccharides derived from bacteria or mammalian tissue cells caused temporary depression of properdin level, while small doses caused transient increases(6-10). It has also been observed that alterations in properdin levels and resistance to infections(11,12), to roentgen irradiation(13), and to hemorrhagic shock(14,15) can be induced by these high molecular weight polysaccharides. Studies in experimental animals also suggest a relationship between serum properdin levels and growth of transplantable tumors. Palm(16) found that rats treated intensively with zymosan, an agent known to influence properdin levels *in vivo*, became temporarily receptive to heterotransplants of the human epidermoid cancer cell line HEp #3. Herbut and Kraemer(17) reported similar results using their human colon adenocarcinoma HR 132 in rats, and postulated that the properdin system might be one of the natural defense factors against neoplasms. Bradner and co-workers (18) found that the transplantable mouse sarcoma S 180 grew faster in mice treated with large doses of zymosan which depressed the properdin levels of these mice, but less well in mice treated with a small dose of zymosan which elevated their properdin levels. Neither these animal studies nor the present clinical

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experiments have demonstrated that properdin has any tumor-inhibitory effect. They merely indicate that conditions which alter serum properdin levels also affect growth of homologous or heterologous transplants. Factors other than properdin may also have been affected by such experimental procedures.

In view of these observations, it seemed of interest to determine whether serum properdin levels differed in the human volunteers who accepted or rejected cancer cell homografts. The present report shows that there are marked differences between the properdin levels of the cancer patients and the normal persons and that a parallelism exists between serum properdin levels of humans and their ability to reject cancer cell homografts.

Methods and materials. The cells (HEp #1, HEp #2, HEp #3, HeLa, HS #1, J-111, Chang's conjunctival cell) implanted into the 2 groups of recipients had been cultivated for long periods (up to 5 years) in tissue culture and/or by passage through cortisone-treated rats or hamsters following their original isolation from human cancer or normal tissues. Although Chang's conjunctival cells were of normal origin they now exhibit the cytologic characteristics of neoplasia(20) and are not considered separately from cells of cancer tissue origin in this paper. The various cell lines were tested in approximately the same proportions in the 2 groups of recipients. The growth of these several types of cells on homotransplantation has been described in a preliminary communication(1) which also gives references to the literature concerning each cell type. *Titration of properdin.* At least one blood specimen from each volunteer was collected for properdin assay on the day of inoculation (day 0) or 14 days thereafter (day 14). From several of the volunteers serial specimens were obtained between day 0 and day 42 or later. After separation from clots, serums were stored for periods up to 6 months at either 5°, −20° or −70°C. Studies in these laboratories have shown that serums could be stored satisfactorily under these conditions. All properdin titers were determined by the zymosan assay(21) employing the same reagents and personnel. Titrations on serums from cancer patients and normals were always run simultaneously. Properdin was titered in at least one serum from each person after the serum had been centrifuged at 35,000 g for 2 hours at 2°C because it has been previously shown(7) that certain serums contain high molecular weight polysaccharide complexes which interfere with the zymosan assay of properdin and which can be removed by ultracentrifugation. This phenomenon was also observed in the present study. An uncentrifuged specimen of each serum was tested simultaneously. Tests were done under code and recorded before correlation with clinical data. Units of properdin/ml of serum are expressed as U. From 1U to 2U is considered a low level of properdin, 3-4U low normal, 5-10U normal, and 12U or greater is considered high.

Results. All 44 serums from the 38 normal volunteers had detectable properdin and gave identical titers before and after centrifugation at 35,000 g. Only 36 of the 53 serums from cancer patients had detectable properdin (11 of the 17 individuals) and 14 of these showed a 1 to 2U drop in titer after centrifugation. The nature and significance of this phenomenon in cancer patients are under investigation. Properdin titers presented hereafter are those determined after centrifugation. However, presentation of the titers of uncentrifuged serums would not materially influence the results.

Serial serum samples from individual persons showed little variation in properdin titer. Among 13 normals from whom serial serums were tested, 5 showed a slightly lower titer 7 to 14 days after implantation than pre-implantation serums. However, no titer went below normal levels and the slight falls were not related to severity of local erythema or induration, or type of cell implanted. In the cancer patients there was no consistent change in properdin titers. One cancer patient had a transient rise from 1U to 4U at a time when an implant was dormant but other cancer patients showed either no change or progressive fall. Since the fluctuations of properdin level during the experimental period were negligible and were unrelated to the phenomenon being studied, all data presented hereafter refer to a single properdin titer in

TABLE I. Distribution of Serum Properdin Titers in Normal Adult Humans and Patients with Advanced Neoplastic Diseases. Entries indicate number of individuals.

Groups	No. of persons	Serum properdin (U/ml) <1	1	2	3–4	5–6	7–8	9–10	11–12
All normals	38			2	4	9	7	13	3
All cancer patients	17	6	3	5	3				
Epidermoid cancer of:									
Bladder, cervix, vulva	5	2	2		1				
Pharynx, larynx, lung	4	1		3					
Adenocarcinoma of:									
Ovary, uterus	2	1		1					
Rectum, sigmoid	2	2							
Lung	1				1				
Sarcomas:									
Melanoma	1		1						
Reticulum cell sa.	2			1	1				

each patient. When serial specimens were tested. the serum collected on or closest to day 14 is recorded.

Comparison of the 2 groups of volunteers (normals and cancer patients) revealed marked differences in properdin levels (Table I). The 38 normal volunteers had a mean properdin level of 7U. and none was below 2U. The 17 cancer patients averaged less than 2U and 6 were < 1U. Overlap between the two groups occurred only at 2 to 4U. Diagnoses are indicated in Table I, but the series is too small to permit any conclusion regarding relationship of type of cancer to properdin levels.

There was also little overlap between the normal and cancer groups in their receptivity to homotransplants. Among the 17 cancer patients, only 2 failed to show nodule formation due to growth of the implanted cells as demonstrated by biopsy. Both of these patients had properdin titers of 4U. Spontaneous regression occurred in 4 patients (properdin titers 4U, 2U, < 1U, < 1U) whose implants were not excised but this started comparatively late (third to fourth week after implantation) and was characterized by mononuclear cell infiltration rather than acute inflammatory reaction. Five cancer patients had persistent or recurrent growth of the implanted cells at day 28 or later. In 3 of these, growth of implants continued throughout the period of the patients' survival (6 to 10 weeks) and one, whose properdin titer was < 1U, had metastases of the homotransplanted cancer cells from the implant site to regional nodes. In contrast, among the 38 normal recipients the transplants elicited a marked local acute inflammatory reaction which persisted until the material was completely resorbed, usually about 3 weeks. Biopsies showed that only about half of the normal recipients had implanted cells remaining at 14 days, and in these the implanted cells were degenerating and surrounded by an intense inflammatory reaction with polymorphonuclear as well as mononuclear cell infiltration. Only one normal volunteer had demonstrated cancer cells remaining after 14 days. His properdin titer was 2U, the lowest in the normal group. Table II and Fig. 1 and 2 summarize these data and show the parallelism between properdin levels and ability to reject the implanted cells.

Whether considering HEp #3 cells alone (Fig. 1) or all cell types combined (Fig. 2), almost all biopsies taken at 6 to 10 days after implantation contained recognizable cells of

TABLE II. Properdin Levels and Cancer Cell Homografts in Man.

	Reaction to homotransplant (see text for criteria of growth & rejection)			
	Growth with metastases	Growth	Rejection	Serum properdin levels
Cancer patient volunteers	1	14	0	Low
	0	0	2	Normal (3U-10U)
	0	0	0	High
Normal volunteers	0	1	0	Low
	0	0	33	Normal (3U-10U)
	0	0	4	High

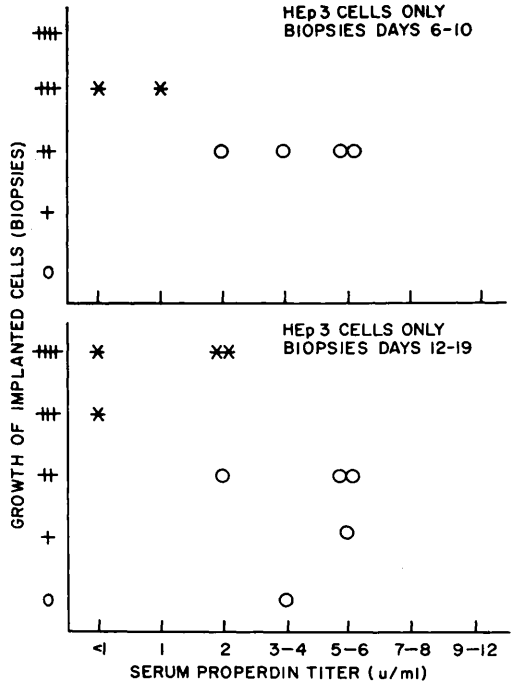

FIG. 1. Relationship between serum properdin and microscopic characteristics of homotransplants of HEp #3 cells at 1 wk (upper graph) and 2 wk (lower graph) after subcut. implantation. Asterisks indicate recipients with advanced cancer. Open circles indicate normals. Biopsies graded as follows: 0, no implanted cells remain, inflammation only; +, few of the implanted cells remain, much inflammation; ++, implanted cells in moderate numbers but inflammatory reaction predominates; +++, considerable numbers of healthy-appearing implanted cells with slight inflammatory reaction, or few cells with no inflammation; ++++, excellent growth of implanted cells without inflammation.

the implanted type. The biopsies from cancer patients showed little or no inflammatory reaction and it was these same patients who had low properdin titers. After another week (bottom half of Figs. 1 and 2) the divergence between the 2 groups was much more marked. Definite propagation of the implanted cells had occurred in the cancer patients with low properdin titers, while regression and marked inflammatory changes were characteristic of the normal recipients with high properdin titers. The number of entries in these graphs does not equal the number of volunteers studied because some individuals were not biopsied within the indicated time periods and some had simultaneous biopsies of 2 implants of different cell types.

Table II simplifies the statement of correlation by making a single evaluation of homotransplant behavior in each *individual*. A recipient is tabulated as supporting "growth" of transplants if some type of implanted cell caused progressive growth of a nodule for at least 2 weeks with histologic demonstration of healthy appearing cells of the implanted type without acute inflammatory reaction or monocytic infiltration. (In 3 cases in which longer follow-up was impossible biopsies taken between days 6 and 9 were accepted as evidence of implant growth because the implanted cells were propagating without evidence of regressive changes.) A recipient is tabulated as showing "rejection" if there was no gross evidence of growth of any implant at any time, or if there was gross and histologic evidence of regression on or before day 14.

Discussion. The foregoing experiments show that the patients with advanced cancer had low properdin titers and that these same patients readily accepted cancer cell homo-

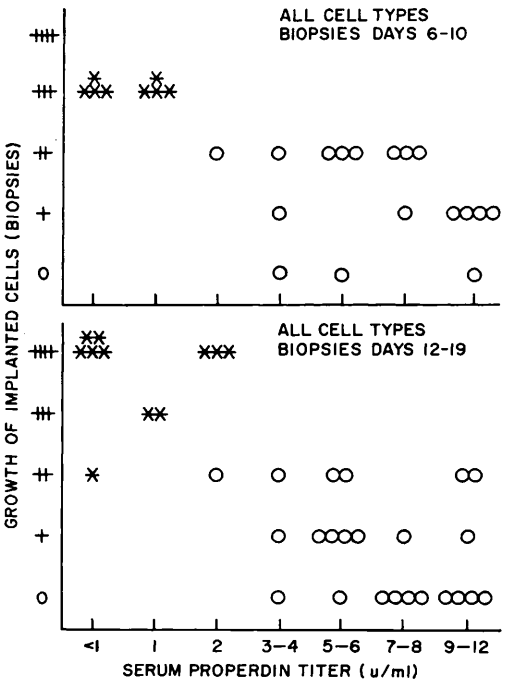

FIG. 2. Relationship between serum properdin and microscopic characteristics of homotransplants of various types of cultivated human cells (HEp #1, HEp #2, HEp #3, HeLa, HS #1, J-111, Chang's conjunctiva) at 1 wk (upper graph) and 2 wk (lower graph) after subcut. implantation. See Fig. 1 for explanation of symbols.

grafts. In addition, there was evidence of a correlation between serum properdin levels and ability to reject homotransplants of cancer cells. There is however no evidence to indicate that this relationship is causal. It must also be emphasized that there is no evidence that the ability to reject homologous cancer cell implants reflects a defense mechanism which controls the occurrence or growth of spontaneous cancer.

Obviously the 2 study groups differed in many respects other than their serum properdin levels. Almost all of the cancer patients had disseminated disease and were cachectic and terminal. The normal volunteers were all male and 6 were Negro. Half of the cancer patients were female and one was Negro. No person in the normal group had any evidence of acute or chronic disease. Median age was 35 years for the normals and 62 for the cancer patients, but there was no relationship between age and properdin levels or growth of implants within either group.

However, none of the cancer patients had received treatment within 3 months of implantation with x-ray, alkylating agents, steroid hormones, ACTH, or metabolic antagonists. None of the patients had an abnormal differential leukocyte count, and only 2 had primary reticuloendothelial system neoplasia. They responded with leucocytosis and fever to bacterial infections. Mobilization of inflammatory cells in response to an irritant was demonstrated in one patient (who had a properdin titer of 1U and whose implanted cells metastasized) after application of croton oil to a scarified area of skin. This area rapidly became red and hot and, as shown by biopsy 6 hours later, was infiltrated by polymorphonuclear leukocytes. All of the cancer patients produced antibodies to viruses administered as experimental therapeutic agents shortly before, during, or after the implant studies. Time of appearance and titer of antibodies seemed normal, but direct comparison of normal persons is not possible. Studies demonstrating antibody production by similar groups of cancer patients have been published(3,4). Complement titers on a similar group of cancer patients showed no major deviation from normal range(5). None of these patients had uremia or gross hepatic dysfunction at the time of these studies. Serum protein partitions on 6 of the cancer patients showed that 4 had hypoproteinemia and hypoalbuminemia and one had a slight hyperglobulinemia, but properdin titers and acceptance of transplants were independent of these protein values.

Since none of the factors which are thought to be related to body defense mechanisms were demonstrably deficient in the cancer patients, properdin remains as the one factor considered to be one of the natural defense mechanisms which thus far has been shown to be deficient in these patients.

There is as yet no explanation for the low properdin levels observed in cancer patients. None of the cancer patients in this study had recently received x-ray or anti-cancer chemotherapy, and infection seems inadequate to explain the properdin depressions. While one patient with a very low properdin titer had a chronic urinary infection (Pseudomonas and mixed coliform bacteria) and another had subcutaneous abscesses (mixed staphylococci, streptococci, and diphtheroids), the others had no obvious bacterial infection. Several patients had experimentally induced virus infections during these studies (West Nile, Mengo, and Semliki Forest viruses) but the serial specimens seemed adequate to exclude viremia, antibody production, or clinical reaction as possible explanations for the low properdin levels. An increased frequency of low properdin titers in cancer patients was also observed by Rottino and Levy(19) and Isliker(22), but not by Hinz(23). The fact that administration to mice of polysaccharides from normal and neoplastic mouse tissues(8) can depress serum properdin levels suggests the possibility that a large mass of cancer tissue may of itself absorb properdin or liberate into the circulation tissue polysaccharides which combine with properdin, thus depressing properdin levels.

Summary. Low levels of serum properdin were found in patients with advanced cancer whose ability to reject cancer cell homografts was defective. Normal properdin levels were observed in normal persons who rejected cancer cell homografts promptly. Investigation

of cellular and humoral defense mechanisms other than properdin has thus far revealed no differences which might explain the impaired rejection of implanted cells by the cancer patients.

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Preparation and Inactivation of Purified Poliovirus: Comparison of Vaccines Derived from Mahoney and Parker Poliovirus.* (23551)

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A great many studies related to preparation of poliomyelitis vaccine have been made, but little work has been done with poliovirus freed from the impurities (nutrient substances, cell components and salts) that accompany the virus in tissue culture fluids.

The advantages inherent in a vaccine derived from purified virus led us to undertake the purification of poliovirus and to study the properties of the purified virus as it became available. With respect to vaccine preparation, we were interested especially in the inactivation of the virus by formaldehyde and the immunogenic properties of the non-infective product.

Methods. Preparation of purified poliovirus. Our studies of the isolation of poliovirus led to a modified version of the Schwerdt and Schaffer procedure(1-3). Details of the isolation have been described(4,5). We have retained the first precipitation step at pH 4.0 to 4.5 with methanol in the presence of celite at 5°C. The filter cake is transferred to a column and eluted with 2% NaCl in 1% phosphate buffer at pH 7.0. This column elution results in a 300-fold increase in virus concentration. The butanol emulsification

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