

Impaired Host Defense against XC Cell-induced Tumors in Thymectomized and in Bursectomized Chickens¹

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SUMMARY

XC cells originally derived from the tumor of a rat previously inoculated with the Prague strain of Rous sarcoma virus were used to induce tumors in chickens surgically thymectomized or bursectomized in the newly hatched period. Thymectomized chickens had a significantly higher incidence of tumors, larger tumors, and a higher tumor mortality, compared with control chickens, when both groups were given 5×10^6 XC cells into the wing webs. Bursectomy could significantly influence the tumor size only. It appeared that the capacity of XC cells to induce tumors and the growth of such tumors were subject to immunological influence, with the thymus playing a major and the bursa of Fabricius a minor role under the conditions used.

INTRODUCTION

The immune system in chickens seems unique, since this species possesses 2 distinct organs that regulate immunological functions. The bursa of Fabricius controls humoral immunity (2, 3), and the thymus controls cell-mediated immunity (2). Thus, removal of the bursa or the thymus results in impaired humoral or cell-mediated immunity, respectively (2, 3). Therefore, this animal model would offer a unique opportunity to study the separate influences of cell-mediated immunity and of the antibody-forming system on tumor development *in vivo*.

Such studies have demonstrated that thymectomized chickens infected with the Carr-Zilber strain of Rous sarcoma virus (14) have significantly higher mortality and incidence of metastases, compared with controls, and also a somewhat higher tumor incidence. When chickens are thymectomized in the newly hatched period and infected with reticuloendotheliosis virus, strain T, systemically or locally, a significantly higher tumor mortality is observed in the thymectomized animals, and normally regressing local tumors grow progressively in thymectomized birds (10, 21). Thymectomy has been reported not to change the incidence of erythroblastosis, or of an avian leukosis, induced by RPL 12 virus (12).

Thus, while data in some tumor systems support the

concept of a "surveillance" function for cell-mediated thymus-dependent immunity, others do not.

Bursectomy data in certain tumor models also support the concept of host protection by the bursa-dependent antibody-forming system, while others fail to do so. Hormonal or chemical (cyclophosphamide) (5, 7) bursectomy reportedly does not affect the incidence or development of tumors induced by the Bryan strain of RSV² (11). Surgical or chemical (cyclophosphamide) bursectomy in the newly hatched period has been demonstrated to significantly increase tumor mortality and result in progression of normally regressing tumors in chickens infected with reticuloendotheliosis virus, strain T (10, 21). It has also recently been found that a chemical carcinogen-induced transplantable tumor line grows at a more rapid rate in bursectomized chickens (6). Bursectomy in newly hatched or young chickens can prevent the development of avian leukosis induced by RPL 12 virus, demonstrating bursal cells as the target cells for this virus (12, 13), but not elucidating the function of the bursa in tumor control in general.

In an attempt to shed further light on the controversial issue of "immunosurveillance" by cell-mediated immunity and by the antibody-forming system in tumor development, we decided to use tumor cell line XC, which, to our knowledge, has not previously been studied in this respect. XC cells are derived from a rat inoculated with the Prague strain of RSV (18). These cells contain no detectable RSV; however, they contain the RSV genome. RSV can be recovered from XC cells by cocultivating them with chicken embryo fibroblasts. Inoculation with XC cells or with the supernatants of the mixtures of XC cells and chicken embryo fibroblasts can induce tumors in chickens. In both cases, infectious RSV is formed and released when virogenic XC cells come in contact with sensitive chicken cells (19, 20).

By using surgical thymectomy and bursectomy in the newly hatched period in combination with the administration of XC cells, we have been able to demonstrate that both of these manipulations, and consequently both cell-mediated immunity and the antibody-forming system, influence the development of XC cell-induced tumors in chickens.

MATERIALS AND METHODS

XC Cells. XC cells (obtained from Dr. A. Girardi, Wistar Institute, Philadelphia, Pa.) were grown in Eagle's minimum

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² The abbreviation used is: RSV, Rous sarcoma virus.

essential medium (Grand Island Biologicals, Grand Island, N.Y.) enriched with 10% fetal calf serum (Grand Island Biologicals), 100 units of penicillin per ml, 100 µg of streptomycin per ml, and 0.15% of sodium bicarbonate. The cells were kept in a water-jacketed CO₂ incubator (Hotpack Corporation, Philadelphia, Pa.) with CO₂ tension set at 5%.

Chickens. White Leghorn chickens eggs (WC line, Hy-Line International, Des Moines, Iowa) were incubated and hatched in a Jamesway egg incubator (Butler Manufacturing Co., Fort Atkinson, Wis.) under standard conditions. The chickens were kept in a thermostatically controlled brooder for 31 days after inoculation with XC cells, with free access to feed and water.

Surgical Manipulations, Tumor Inoculation, and Evaluation of Data. The chickens were surgically thymectomized or bursectomized within 24 hr of hatching using standard techniques (12). Twenty-six thymectomized, 25 bursectomized, and 22 control chickens were used in the study. The control group was not sham operated, since separate studies have shown no difference between sham-thymectomized or sham-bursectomized and normal animals in terms of take, growth rate, or mortality of XC cell-induced tumors. The surgically treated groups of chickens and the control group were inoculated into the wing webs with 5 × 10⁶ viable (trypan blue-excluding) XC cells on the day after surgery. The length and width of tumors that developed within 31 days were measured (in mm) twice weekly with a caliper. The observations could not be carried further because of the high tumor mortality in the thymectomized group. The size of the tumors was expressed as the product of these measurements. Tumor incidence and mortality were recorded. These data were compared statistically. Fisher's exact test for 4-fold tables was used to compare tumor incidence and animal mortality, and the analysis of variance was used to evaluate tumor growth in the 3 groups of animals.

RESULTS

Effect of Thymectomy or Bursectomy on the Incidence of Tumors in Chickens. At the end of the 31-day period, 25 of the 26 thymectomized chickens (96%), 16 of the 25 bursectomized chickens (64%), and 14 of the 22 controls (63%) had developed tumors. The incidence of tumors in thymectomized chickens as compared with normal controls was significantly higher ($p < 0.02$). However, surgical bursectomy alone did not seem to have any effect on tumor frequency (Table 1).

Table 1

Incidence of tumors in thymectomized, bursectomized, and control chickens inoculated with XC cells

Treatment	No. of chickens	No. of chickens with tumors	%
Thymectomy	26	25	96 ^a
Bursectomy	25	16	64
Control	22	14	63

^a Significantly higher ($p < 0.02$) when compared with controls (Fisher's exact test).

Effect of Thymectomy or Bursectomy on Tumor Mortality. Thymectomy or bursectomy had no effect on the mortality of the tumor cell-inoculated chickens during the 1st 27 days. A higher percentage of deaths in thymectomized chickens was observed (22%), compared with controls (4%), on the 28th day. By the 29th, 30th, and 31st day, significantly higher mortality in thymectomized chickens as opposed to controls was observed ($p < 0.01$, $p < 0.002$, and $p = 0.02$, respectively). The deaths were associated with large tumors, not with general poor health of the thymectomized animals. Bursectomy had no effect on mortality during the observation period (Chart 1).

Effect of Thymectomy or Bursectomy on Tumor Size. Prior to the 15th day, tumors were diffuse and difficult to measure. After that, more spherical, well-demonstrated tumors developed. The mean tumor size of bursectomized, thymectomized, and control groups is presented in Chart 2. It can be seen that there are no clear-cut differences in

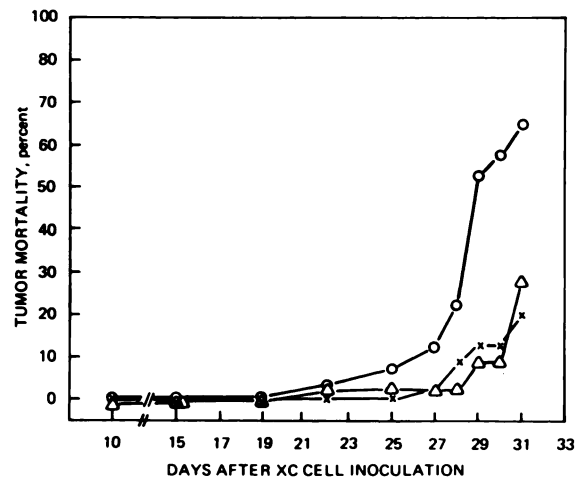


Chart 1. Cumulative mortality of thymectomized (O), bursectomized (x), and control (Δ) chickens inoculated with XC cells.

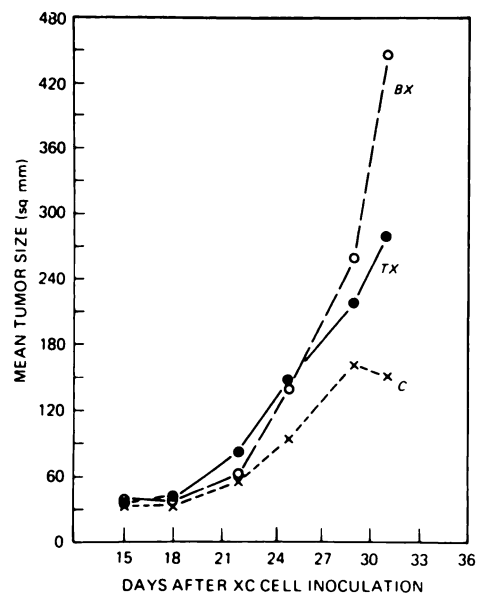


Chart 2. Mean tumor sizes of thymectomized (TX), bursectomized (BX), and control (C) chickens inoculated with XC cells.

mean tumor size during the 1st 22 days of inoculation. From the 25th to the 31st day, the difference in tumor size among the groups became apparent.

The analysis of variance was used to compare tumor sizes in the different groups. Although thymectomized animals had considerable larger tumors than controls (Chart 2), this method of comparison did not demonstrate a statistically significant difference between the groups. However, the tumors in bursectomized animals were significantly larger ($p < 0.01$) than in the controls at the end of the observation period (31st day).

DISCUSSION

We have demonstrated that thymectomized chickens inoculated with XC cells have a higher incidence of tumors, larger tumors, and a higher mortality rate compared with control chickens. Bursectomized chickens also exhibited significantly larger tumor sizes; however, there were no clear-cut differences in mortality or incidence of tumors with the experimental protocol used. In order to obtain a high tumor-take frequency, XC cell inoculation was performed on the day after hatching, when the immune system of the recipients was still somewhat immature. A difference in tumor incidence might be demonstrable also in bursectomized birds, if the animals are inoculated at a later time. The thymectomy results showed that the thymus may play an important role in host protection in XC cell tumor growth and development in chickens, and lend support to the involvement of the thymus and thymus-dependent immunity in surveillance (1, 10, 17, 21) in this tumor model. Although the bursa of Fabricius does not seem to contribute as much as the thymus in this instance, it does influence tumor growth rate and consequently also plays a role in host protection. It should also be noted that surgical bursectomy alone in the newly hatched period does not lead to absence of antibody-forming and immunoglobulin-producing capacity (16, 23). Studies with bursectomy methods more severely affecting humoral immunity (5, 7) are necessary to evaluate further the extent of the contribution by the antibody-forming system in the control of XC cell tumors.

The influence of thymectomy on tumor induction by the Carr-Zilber strain of RSV in the chicken has been reported by Radzichovskaja (14). She observed that thymectomy significantly increased mortality and frequency of metastases and also noted a somewhat higher tumor frequency and tumor growth rate in thymectomized chickens. When normal Japanese quail were inoculated with the Schmidt-Ruppin strain of RSV, the tumor regression rate was 90 to 100%. In contrast, the thymectomized groups had only a 0 to 30% regression rate (24). Thus, thymectomy lowered the host's ability to regress tumors, presumably by impairing cell-mediated immunity. Comparing our present data on the effect of thymectomy on the growth of XC cell tumors with those on other RSV-induced tumors, it appears that the virogenic XC cells respond in an essentially similar way in hosts with impaired cell-mediated immunity. Although surgical thymectomy without adjunct treatment does not abolish cell-mediated immunological functions, it seems to have impaired cell-mediated immunity sufficiently to permit in-

creased tumor development in this study and in those quoted above.

Previous results in RSV-infected chickens failed to show any effect of bursectomy (11, 15) and agammaglobulinemia (11) on tumor incidence or growth rate, or on tumor size, leading those authors to the conclusion that antibodies do not play a significant tumor-enhancing role under the conditions used (11). The data also failed to demonstrate a host-protective role for the antibody-forming system. Inoculation of Japanese quail, which had been bursectomized when 1 week old, with the Schmidt-Ruppin strain of RSV also had no effect on tumor development (24). It was suggested by the latter authors that humoral antibodies did not play an important tumor-enhancing role in Rous sarcoma in birds under the conditions they used. However, while the quail data led the investigators to a conclusion similar to that obtained in the study on chickens, they are somewhat difficult to interpret, since the bursectomy syndrome has not been well defined in the quail, and varies remarkably from species to species (22). Due to the negative reports on effect of bursectomy using 2 strains of Rous sarcoma, we decided to use a different agent, namely XC cells, carrying the genome of the Prague strain of Rous sarcoma. Using this approach, we were able to show a difference in tumor growth rate between surgically bursectomized and control birds. Surgical bursectomy alone, in our hands and those of others, does not significantly reduce immunoglobulin levels, but markedly depresses the capacity to respond to certain antigens. Whether the different results obtained in our study and in the earlier ones reflect a difference between Rous sarcoma strains or depend on other factors, is unknown.

The involvement of the thymus and the bursa of Fabricius in the regulation of development of other avian RNA tumor viruses in chickens has been documented. Thymectomy and bursectomy significantly increase tumor mortality in chickens after systemic and local administration of reticuloendotheliosis virus over that found in sham-operated controls (9, 10, 21). In addition, most of either thymectomized or bursectomized chickens develop progressively growing wing-web tumors when a virus dose is used, which leads to regression in most unmanipulated controls (9, 10). Regression of local tumors can be accomplished by repeated administrations of immune serum. The curative effect is not dependent on antiviral antibodies, as the antiserum is effective after repeated absorptions with reticuloendotheliosis virus (4, 8). This strongly suggests that the component active in immune serum is directed against tumor-associated transplantation-type antigens.

The data presented here, combined with the results from the avian reticuloendotheliosis studies, and with a recent finding that the growth rate of a benzo(α)pyrene-induced transplantable tumor line is considerably faster in bursectomized than in control birds (6), demonstrate that humoral immunity contributes to host protection. A similar finding in these different tumor systems motivates the proposition that the host-protective influence of the antibody-forming system in tumor development is a general phenomenon in host defense against tumors. The effectiveness of this line of defense may vary with the intrinsic properties of the

tumors, such as their proliferation rate, antigenicity, and their ability to use escape mechanisms from immunosurveillance.

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