Activation of Complement in Quails Bearing Rous Sarcoma Virus-Induced Tumors

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ABSTRACT. The concentration of serum C3 in quails bearing tumors induced by Rous sarcoma virus (RSV) was elevated in parallel with tumor growth, whereas serum C3 levels in quails inoculated with avian leukemia virus, which lacks transforming activity, showed a pattern similar to that in mock-infected quails. C3 deposition was also observed in almost all tumor cells at the tumor developing stage. These findings obtained in vivo suggest that the cells transformed by RSV activated the C3 on their surfaces. — KEY WORDS: ACP, complement, Rous sarcoma virus.


Host responses to tumors induced by Rous sarcoma virus (RSV) in Japanese quails have been shown to involve cell-mediated immunity to tumor-associated antigens and humoral factors [3]. We also reported that quails bearing RSV-induced tumors showed a relatively high titer of the alternative complement activity [18]. An association between the complement and tumor development has been suggested in several instances. In patients with malignant lymphoma, Hodgkin's disease and myeloma, high levels of complement activity have been reported [4, 12]. The size of spontaneous lymphoma in AKR mice, which are genetically deficient in C5, was also reported to be reduced by the administration of C5 [9]. These findings indicate that the complement plays an important role in tumor resistance, however, to our knowledge, experimental confirmation of this possibility has not been reported. Previously, we showed that quail cells transformed by RSV activated the homologous quail complement in vitro via the alternative complement pathway (ACP) [8] by using a monospecific antibody to quail C3 [6]. In view of these results, we undertook the present study to measure serum C3 levels in quails during development of RSV-induced tumors.

Japanese quails and quail eggs were obtained from the Nippon Institute for Biological Science, Tokyo. Seven-day-old embryos were used for primary cell culture, and 5- or 6-week-old quails were used for virus infection. Primary quail embryo (QE) cells were cultured in Dulbecco's modified Eagle's minimum essential medium supplemented with 10% tryptose phosphate broth and 5% newborn calf serum as a growth medium and used for titration of virus infectivity.

To investigate the relation of tumor development to the serum C3 levels, 48 quails were inoculated subcutaneously (sc) with 0.1 ml of a stock solution of subgroup A, Schmidt–Ruppin strain of RSV (SR-RSV) having an infectivity titer of 1.2 × 105 focus forming units (FFU)/ml, and 32 quails were mock-inoculated as controls. Three to 9 quails were killed at 2- to 4-day intervals for 18 days after virus inoculation, and their tumor sizes were measured. At the same time, serum was obtained by cardiac puncture, and C3 concentration in serum was assayed by rocket immunoelectrophoresis using anti-quail C3 serum [7] and expressed relative to the C3 concentration of pooled serum from 50 normal quails at 7 to 8 week-old (defined as 1.0).

Tumors began to develop at day 5, reached a maximum size at day 11, and then regressed (Fig. 1b). Significantly higher levels of serum C3 were observed in the tumor bearers also on day 11 than in the controls (p<0.05) (Fig. 1a). C3 concentrations in tumor bearers seemed to be roughly correlated with their tumor sizes, although serum C3 concentrations in the control quails were randomly distributed over a wide range (1.25 ± 0.65).

Because there was a large variation in C3 levels in normal quail serum, a correlation between serum C3 concentration and tumor size was investigated individually. Nine quails were inoculated with SR-RSV having the same titer as described above, and serum C3 levels were compared with tumor sizes. The results are shown in Fig. 2a. Three quails were complete regressors, 3 were incomplete regressors and the remaining 3 were progressors. In all cases, an increase in tumor size was accompanied by an increase in the amount of serum C3. After the tumors reached their maximum size, serum C3 began to decrease with tumor regression in both the complete and incomplete regressors. In the progressors, serum C3 levels were tended to increase rather than decrease.

C3 is consumed by the activation of complement in the local region. However, C3 is very rapidly produced in compensation for it, and serum C3 level as well as the complement activity becomes higher than usual. The high level of serum complement is often observed in cases who have inflammations or malignant lymphomas. Therefore, the correlation between high C3 level in quail serum and the tumor size suggested the activation of complement by the tumors.

To investigate whether the increase in serum C3 was due to activation of complement by transformed cells or by the virus, we examined serum C3 levels in five quails of the same age inoculated sc with 0.1 ml of a stock solution of avian leukemia virus (RAV-1) having infectivity titer of 2 ×

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$10^3$ infectious units (IU)/ml as determined in QE cells by interference with SR-RSV, which belongs to the same subgroup of SR-RSV but which lacks transforming activity. The blood was collected by partial bleeding from the wing web vein at 2- or 3-day intervals from 5 to 18 days post inoculation. As shown in Fig. 2b, serum C3 levels varied widely after virus inoculation, and showed inconsistent patterns. Thus, higher serum C3 levels appeared to occur only in tumor-bearing quails and not in RAV-1-inoculated quails.

Using the same serum samples as shown in Fig. 2a, C3b conversion rates were examined comparing with C3 levels by disc agarose gel isoelectrofocusing (DAIEF) as described previously [6]. Serum C3 levels were clearly higher in the tumor bearers as described above. However, in most of serum samples the conversion of C3 to C3b was not observed at any stage (data not shown).

To investigate the possibility of complement activation by the tumor cells, C3 deposition on tumor cells was examined by an indirect immunofluorescence technique. The tumor masses induced by SR-RSV were obtained from wing webs of quails and frozen with n-hexane in a dry ice-acetone bath. Sections of $3 \mu m$-thickness were made with a cryostat and fixed with acetone at $-20^\circ C$ for 20 min. The tissue sections were incubated at $37^\circ C$ for 30 min with 1:30-diluted fluorescein isothiocyanate-labeled anti-rabbit IgG (Cappel Lab. Inc., PA) that had been preabsorbed twice by QE cells. Abundant C3 deposition was observed in the tumor cells, especially in tumor cells infiltrating into muscles (Fig. 3a, c). No specific fluorescence was observed on lymphocytes or on normal muscle cells.

The close positive correlation between serum C3 level and tumor size in tumor-bearers, as well as the abundant C3 deposition in tumor tissues, indicates that the activation of homologous complement leading to C3 deposition on their surface by RSV-induced tumors occurred in vivo, as it did in the in vitro experiments [8]. The increased serum C3 levels seemed to be associated with the RSV-induced transformation but not with the virus particles per se, because no consistent patterns on the increase in serum C3 level was observed in quails infected with RAV-1. These results were well in accordance with our previous in vitro findings that the C3 deposition on the primary quail cells were observed on SR-RSV-transformed cells but not on RAV1-infected cells regardless of the production of progeny viruses, and that the cells infected with temperature-sensitive mutant from SR-RSV only activate complement when they were morphologically transformed [8].

The activation of complement via the classical complement pathway leading to lysis of RNA viruses has been reported [1, 2, 5, 13, 16]. This activation was reported to be caused by virus particles per se. On the other hand,
our previous in vitro study [8] and present study indicated that the activation of autologous complement was caused by RSV-induced quail tumor cells via the ACP. The significance of complement activation via the ACP in nonspecific immunity, especially at an early stage of infection, has been indicated by several investigators [10, 15]. However, the activation of homologous complement via the ACP has been reported in only a few instances, such as in spontaneous tumors [11, 14]. The present system of homologous complement activation both in vivo and in vitro by RSV-transformed cells provides a unique model to investigate roles of complement in tumor immunity.

The ratio of C3b concentration in comparison with C3 assayed by DAIF was not increased concomitant with tumor growth. The lack of correlation between the C3b level and tumor size might be due to the rapid catabolic rate of C3b, clearance of C3b from the serum by deposition on tumor tissues, or rapid hyperproduction of C3 following complement activation by tumor cells. Serum concentrations of C3d in patients with malignant lymphoma were reported to be elevated [4]. However, quail C3d was not well defined by the anti-quail C3 serum, because anti-quail C3d serum is not yet available.

Histopathological examinations revealed edema and...

Fig. 2. Temporal changes of serum C3 in individual quails inoculated with SR-RSV (a), RAV-1 (b). Serum C3 concentration (---) and tumor size (—) after SR-RSV inoculation are expressed as in Fig. 1. The quails were placed in one of three groups (regressors, incomplete regressors and progressors) according to the type of tumor growth.

Fig. 3. C3 deposition on tumor cells in vivo (indirect immunofluorescence technique). Abundant C3 deposition was observed in the mass of tumor cells (a) and tumor cells infiltrated into muscles (c). Immunofluorescence reaction was not observed in a control in which normal rabbit serum was used as the first serum instead of anti-quail C3 rabbit serum (b).
infiltration of heterophils in tumor tissues induced by RSV at the early tumor developing stage prior to the massive infiltration of lymphocytes [17]. The activation of complement by tumor cells at an early stage could play an important role in inducing local inflammation, such as edema and infiltration of heterophils, which, in turn, would lead to an effective induction of a specific immune response by the lymphocytes.

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REFERENCES