Summary

Evidences that demonstrate or imply the occurrence of DNA-RNA viral interactions in man and animals are reviewed. The concurrent presence of two dissimilar onco-
genic viruses influenced responses both in vivo and in vitro. Cell-mediated immune responses to Marek's disease herpesvirus were altered in specific-pathogen-free chickens inoculated as embryos with an avian leukosis virus. The possible roles of immunological factors in the pathogenesis and control of tumor development associated with DNA-RNA viral interactions remain to be investigated.

Interactions between MDHV and ALV initially were demonstrated in tissue culture. Infection of CEF cultures with RAV-2, an ALV of Subgroup B, rendered the cells resistant to superinfection with MDHV as evidenced by inhibition of MDHV focus formation (12). The interference phenomenon was dose dependent since no influence on focus development was observed in cultures infected with relatively low concentrations of RAV-2. The development of foci was not affected in CEF cultures previously treated with heat-inactivated RAV-2 or RAV-2 plus anti-RAV-2 serum. RAV-2 did not inhibit plaque formation by herpes simplex or vesicular stomatitis viruses. Other tests for the presence of interferon-like substances were negative. In some experiments, interference with MDHV focus formation by RAV-2 was accompanied by enhanced COFAL titers (36), although this response was not observed with RAV-1, an ALV of Subgroup A. Focus formation by a herpesvirus of turkeys was inhibited in CEF cultures preinfected with RAV-2, but not with RAV-1 (W. F. Campbell and J. W. Frankel, unpublished observations). Interference with herpesvirus of turkeys foci by RAV-2 was not accompanied by increased COFAL titers.

It was observed that MDHV focus formation did not occur in CEF cultures inoculated with certain extracts (MDHV-A) of the FFE from chickens with MD and continued cultivation led to the detection of ALV (13). The FFE is the source of enveloped, infectious MDHV (5, 30). Other FFE extracts of MDHV produced foci in CEF cultures and infectious ALV was not demonstrated. MDHV-A and MDHV induced focus formation in chicken kidney cell cultures, although MDHV-A foci were smaller than those produced by MDHV. Anti-MDHV serum neutralized MDHV-A and MDHV in chicken kidney cell cultures. MDHV-A and MDHV also shared a major precipitin antigen.

A flock of LSI-SPF chickens was developed (11) for the study of DNA-RNA viral interactions. Monitoring of the LSI-SPF flock over a period of generations did not reveal the presence of MDHV, ALV, or other avian pathogens. The C/O phenotype (24) was predominant.

Studies were performed in several isolated experimental units to determine the response of LSI-SPF chickens contact exposed to chickens inoculated with MDHV, RAV-2, or MDHV plus RAV-2. The infected donor chickens were introduced into these separate holding areas at 2- to 3-week intervals. Mortality did not occur among LSI-SPF chickens housed for 8 weeks in an MDHV-contaminated environment, but MDHV infection had occurred since specific viremia and neutralizing antibodies developed (11). Gross tumors were not observed at necropsy, although histological examination revealed lymphoid cell infiltration in the visceral organs and peripheral nerves. LSI-SPF chickens contact exposed for 8 weeks to chickens infected with RAV-2 developed specific viremia but were asymptomatic, and tumors were not observed at necropsy. The only condition that resulted in mortality (84%) and gross tumor development (100%) among LSI-SPF chickens was contact exposure for 8 weeks to chickens infected with MDHV plus RAV-2. A similar experiment was conducted in isolators with LSI-SPF chickens exposed to chickens infected with MDHV plus RAV-2 or MDHV alone. Sequential histological examination of vagus nerves revealed massive, focal lymphoid cell infiltration as early as 28 days in the former group, compared to 42 days in the latter (C. O. Prickett, unpublished observations).

Inoculation of embryos with RAV-2 affected the response of newly hatched chickens placed in an MDHV-contaminated environment (13). Embryonated eggs from a specific pathogen-free line (SPAFAS) were inoculated via the yolk sac with RAV-2 or with diluent, and the chickens from each group were placed in a facility containing MDHV-infected chickens. Chickens inoculated with RAV-2 in ovo and contact exposed to MDHV exhibited high mortality (71% by 8

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2 The abbreviations used are: MDHV, Marek's disease herpesvirus; ALV, avian leukosis virus; CEF, chicken embryo fibroblast; RAV-2, Rous-associated virus 2; COFAL, ALV complement-fixing antigen; RAV-1, Rous-associated virus 1; MDHV-A, Marek's disease herpesvirus preparation in which ALV is detected; FFE, feather follicle epithelium; MD, Marek's disease; LSI-SPF, isolator-derived, barrier-sustained specific pathogen-free line; SPAFAS, specific-pathogen-free line; pRNA, polysomal RNA; EB, Epstein-Barr.

Jack W. Frankel

Life Sciences Research Laboratories, Life Sciences, Inc., St. Petersburg, Florida 33710
(weeks) compared with the diluent-inoculated group (42%). MDHV was demonstrated in the FFE of all diluent-inoculated chickens tested during a 3- to 5-week period. RAV-2 interfered with MDHV replication, since MDHV was detected in only 54% of similarly sampled chickens from the RAV-2 plus MDHV group.

A survey showed that in addition to RAV-2, ALV of other subgroups and another avian oncornavirus influenced the response of chickens to MDHV (13). Inoculation of isolator-held LSI-SPF chickens with MDHV plus ALV (Subgroups A, B, C, or D) or reticuloendotheliosis virus resulted in markedly enhanced mortality compared to chickens inoculated with MDHV or the respective oncornavirus alone. Six weeks after inoculation with MDHV plus RAV-1, the mortality was 50% compared to 5 and 6% for MDHV and RAV-1, respectively. Interference with MDHV replication in the FFE accompanied the enhanced mortality. MD antigen was detected during 3 to 5 weeks in the FFE extracts from 23% of the chickens that received MDHV plus RAV-1, compared to 79% of the chickens that were inoculated with MDHV only (M. E. Smith, W. F. Campbell, and J. W. Frankel, manuscript in preparation). Interestingly, the coexistence of particles resembling herpesvirus and oncornavirus were observed during electron microscopic examination of the FFE from chickens inoculated with MDHV and RAV-2 (19).

As noted above, ALV was detected in CEF cultures that had been inoculated with certain FFE extracts (MDHV-A), and focus formation was not observed. Other FFE extracts (MDHV) induced focus formation in CEF cultures in the absence of infectious ALV production. MDHV-A produced a high incidence of early mortality (100% by 3 weeks) among isolator-held LSI-SPF chickens compared to 11 to 18% mortality after inoculation with MDHV (13). MDHV-A and MDHV could not be distinguished from one another in S-line chickens (23) with evidence of naturally occurring ALV infection since both types of FFE extracts produced the symptomatology and tumor spectrum characteristic of MD.

A microcytotoxicity test was developed to characterize cell-mediated immune responses of chickens with MD. Techniques utilized in comparable studies with human tumors (29) were adapted for use in the avian system. MDHV-infected and uninfected CEF cultures were used as target cells to which washed lymphoid cells were added with or without autologous sera. In a representative study, lymphoid cells were obtained from chickens reared for 8 weeks in an MDHV-contaminated environment. The washed lymphoid cells from 80% of these chickens were cytoxic for MDHV-infected target cells (J. R. Mitchen, M. E. Smith, and J. W. Frankel, manuscript in preparation). The cytotoxic effect appeared to be specific since the lymphoid cells were not active on uninfected CEF cultures. Autologous heat-inactivated sera “blocked” the cytotoxic effect. Washed lymphoid cells from 20% of untreated 8-week-old chickens were active on MDHV-infected target cells, but “blocking” was only occasionally observed. For assessment of the influence of ALV on cell-mediated responses to MDHV, chickens hatched from embryos inoculated with RAV-2 were exposed to MDHV for 8 weeks. Washed lymphoid cells from these chickens were cytotoxic for MDHV-infected target cells, but autologous sera failed to block this reaction (13). Addition of these sera to a mixture of cytotoxic lymphoid cells and blocking serum resulted in an inhibition of the blocking phenomenon since MDHV-infected target cell destruction was observed. In this preliminary experiment, the presence of blocking or “deblocking” factors correlated with tumor burden, but this finding is yet to be confirmed and extended.

Significantly higher levels of ALV-specific mRNA were observed in tissues from S-line chickens exposed to MDHV alone or MDHV plus RAV-2 than in corresponding tissues from chickens exposed to RAV-2 or from untreated control chickens (33). Chickens exposed to MDHV plus RAV-2 experienced a higher mortality than those exposed to MDHV or RAV-2. Since RAV-2 is an ALV of Subgroup B and the C/B phenotype has been encountered among S-line chickens (R. Cole, personal communication), it is conceivable that the enhanced ALV-specific mRNA may have represented derepression of endogenous ALV or that MDHV stimulated expression of the indigenous ALV.

Elevated levels of ALV-specific mRNA were present in tumor tissues from isolator-held SPAFAS chickens 4 weeks after inoculation with MDHV(13). Examination of the MDHV inoculum by COFAL, resistance-inducing factor (35), simultaneous detection (39), 60 to 70 S RNA, and radioimmune precipitin (40) assays did not demonstrate the presence of infectious ALV. A DNA probe (26) prepared from RAV-2 60 to 70 S RNA was used to detect the ALV-specific mRNA, and hybridization was analyzed by means of thermal elution from hydroxyapatite (25). The extent of hybridization with the DNA probe was significantly greater with pRNA extracted (1) from tumor tissues of chickens inoculated with MDHV compared with pRNA from tissues of uninfected chickens. Hybridization of the ["H]DNA probe with pRNA extracted from kidneys of MDHV-inoculated chickens ranged from 7.3 to 15.9% at a C t of 2000 to 3000. Direct cultivation of the kidney tumor cells resulted in the production of particles containing 60 to 70 S RNA (W. F. Campbell and J. W. Frankel, manuscript in preparation). Inoculation of SPAFAS CEF cultures with fluids containing these particles did not result in oncornavirus replication. The origin, definition, and possible influence on tumorigenesis of the particles observed in cultivated kidney tumor cells is yet to be determined.

Other studies have demonstrated or implied the occurrence of DNA-RNA viral interactions. Particles possessing biochemical characteristics of oncornaviruses were identified in Burkitt’s lymphoma (27) with which the EB herpesvirus is associated (9, 15, 17, 41, 44). The EB virus also has been implicated in infectious mononucleosis (16, 18, 31). Based on experience with a number of animal systems, a most likely candidate for the induction of leukemia in man is an oncornavirus (4). Seroepidemiological studies showed that infectious mononucleosis occurred prior to acute leukemia (28), suggesting that EB virus may be related to factors involved in the activation of the leukemic process. An increased incidence of herpes zoster, an inflammatory disease caused by a herpesvirus (varicella-zoster virus), was recognized in patients with leukemia (2, 10, 14, 38, 42). Inoculation of guinea pigs with an oncornavirus plus a herpesvirus, which were isolated from guinea pigs, resulted...
in hyperplasia of the spleen and lymph nodes (20, 22). The oncornaivirus alone did not produce this response, but the effect was observed with a herpesvirus that probably contained an oncornaivirus (21). Oncornaivirus expressions were activated in tumors induced by cell cultures transformed by polyoma virus (34). An oncornaivirus was detected in Swiss/3T3 cells transformed by UV-irradiated herpes simplex virus type 2 (8). An RNA-dependent DNA polymerase possessing many biochemical properties analogous to those of known primate and murine oncornaviruses was found to be associated with a simian lymphoid cell line derived from a lymphoma induced by Herpesvirus saimiri, although no herpesvirus could be demonstrated in the cultures (43). Herpes simplex virus type 2 stimulated Rous sarcoma virus-transformed cells (7). Herpes simplex virus type 1 replication was restricted in cells transformed by sarcoma viruses of murine, feline, and avian origin (6). SV40 stimulated DNA synthesis and enhanced murine sarcoma virus replication in mouse embryo cells (3).

There is evidence that RNA viruses other than oncornaiviruses can interact with herpesviruses since herpes simplex virus type 1 replication was restricted in vitro by the presence of poliovirus (37). An RNA virus (live modified infectious bronchitis virus vaccine) significantly increased mortality in chickens with infectious laryngotracheitis (32) with which a herpesvirus is etiologically associated.

The observations that the concurrent presence of 2 dissimilar oncogenic viruses influenced host response is of general interest. The possible roles of immunological factors in the pathogenesis and control of tumor development associated with DNA-RNA viral interactions remains to be investigated.

References

Experimental Models for DNA-RNA Viral Interactions: A Brief Review

Jack W. Frankel


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