Harald zur Hausen

Infections Causing Human Cancer

With a contribution of
James G. Fox, Timothy C. Wang
and Julie Parsonnet

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Preface

For many years I have been tempted to write a comprehensive book on the role of infectious agents in human cancers. Progress has been particularly rapid in this field during the course of the past 25 years, and today we can convincingly report that approximately 20% of the global cancer incidence is initiated or promoted by infectious events. I had admired the task carried out by Ludwik Gross. Since his two-volume publication *Oncogenic Viruses* in 1961, with additional editions in 1970 and in 1983, a number of books have appeared on similar topics, virtually all of them authored by multiple scientists and some of them very heterogeneous in content and structure. For these reasons, I planned to write a book which attempted to develop a more unifying concept and a consistent structure for the individual chapters. Considering the overwhelming magnitude of data, I was sure that I could not undertake this task during my active period as scientific director of the German Cancer Research Center in Heidelberg, and so postponed this for "active retirement". Ultimately, I was pleased that I was able to persuade James Fox from Harvard University to contribute Chapter 10, on *Helicobacter*, as this would have been beyond my personal experience. He immediately consented and jointly with Timothy C. Wang and Julie Parsonnet delivered the chapter in time.

The book is not intended to cover the structure and molecular biology of the agents presented in great detail, but rather aims to concentrate on those aspects that link the respective agents to human oncogenesis. The book should introduce interested colleagues, clinicians, and students to the field, and help to analyze some of the developments that even 20 years ago attracted only minimal attention. Today, this research has culminated in the development of the first – and apparently successful – vaccines for the prevention of specific, common human cancers, cervical carcinomas, and liver cell cancer. Within the book we have tried to provide the readers with an extensive bibliography after each individual chapter, in order to permit further studies on the subject. However, even an attempt to select the most important papers in the field will almost inevitably miss some publications that our colleagues consider as very important. Consequently, I apologize in advance to all of those readers who feel that we did not cover their own or other research areas adequately.

Fortunately, the response on the part of my colleagues was friendly and generous, and they provided helpful suggestions and corrected some of my statements. I am

particularly indebted to George and Eva Klein, Stockholm, for their many extremely helpful comments, to Bernhard Fleckenstein, Erlangen, and Georg Bornkamm, Munich, Vladimir Vonka, Prague, Nikolaus Müller-Lantzsch, Homburg, to Reinhard Kurth, Norbert Banner, and Georg Pauli, all Berlin, and to my Heidelberg colleagues, Frank Rösl, Rainer Schmidt, Lutz Gissmann and Henri-Jacques Delecluse. My secretary, Gudrun Küthe, competently and patiently checked the entire manuscript and corrected initial mistakes. Sherryl Sundell, the Managing Editor of the *International Journal of Cancer*, tried to correct at least some of the "Germanisms" in the language.

A special note of gratitude goes to my wife, Ethel-Michele de Villiers, who not only patiently tolerated two-and-a-half years of evenings and weekends devoted to reading and writing, but also actively contributed by discussing and modifying part of the text. Last, but not least, I would like to mention my granddaughters, Talisa and Johanna, who were to some extent neglected during this period. This is hopefully going to change now.

Harald zur Hausen
Heidelberg, April 2006

1
Historical Review

1.1
The Early Period (1898–1911)

On March 24, 1882 Robert Koch presented his famous lecture at the Physiological Society in Berlin, suggesting that tuberculosis is caused by a bacterium. It was probably this surprising discovery of the infectious etiology of tuberculosis – a disease which until then was not suspected to be caused by an infectious agent – that turned the interest of microbiologists at the end of the nineteenth century towards a possible infectious cause of other chronic conditions, among them cancer. Interestingly, at the turn of the past century, the first positive reports incriminated parasites, liver flukes and *Schistosoma* infections with specific human cancers: in 1900, Askanazy reported a link between *Opisthorchis felineus* infection and liver cancer in the former East Prussia. Only five years later, another report incriminated *Bilharzia* infections (schistosomiasis) in bladder cancer (Goebel, 1905). Goebel mentioned in his paper (without citation) that Griesinger and Bilharz, Zancarol, Kartulis, Harrison, Chaker, Rütimeyer, Scheube, Lortet and Vialleton, Brault and also Albarran had demonstrated previously that chronic schistosomiasis might eventually lead to the development of cancer. Possibly based on these findings, Johannes Fibiger in Denmark reported in the early 1920s the identification of a nematode, *Gangylonema neoplasticum* (initially named *Spiroptera carcinoma*), in rat tumors and drew far reaching conclusions from these investigations also for other types of cancer. For his studies, Fibiger was awarded the Nobel Prize in Medicine in 1926. Unfortunately, his results have not been confirmed by other groups, though an account of the developments leading to his award was published recently (Stolt et al., 2004). Subsequently, at least one other nematode, *Spirocerca lupi*, has been identified as causing esophageal granulomas in dogs, some of them converting into sarcomas (Bailey, 1963).

In those years the evidence for a role of these parasites in human cancer was based exclusively on epidemiological observations and on the findings of clinical studies. Nevertheless – and based on numerous subsequent observations – a panel established in 1994 by the International Agency for Research on Cancer (IARC) in Lyon concluded that there exists sufficient evidence for a role of two parasites, *Schistosoma haematobium* and *Clonorchis viverrini*, in human cancers (IARC, 1994). The same report claimed that there is limited evidence for a carcinogenic function of *Schistosoma japonicum* and for the liver fluke *Clonorchis sinensis*.

The early reports of a link between parasitic infections and human cancers were even preceded by a study of M'Fadyan and Hobday in England. In 1898, these authors published details of the transmission of dog warts by papilloma filtrates known to retain all characterized bacteria identified to that time. Warts, of course, were not considered as "true" tumors, and therefore this publication received little attention. The existence of a "contagium fluidum", which passed filters that would retain all known microorganisms, had been established previously by Iwanowski in St. Petersburg in 1894, and also by Beyerinck in Amsterdam in 1898. Both M'Fadyan and Hobday and Iwanowsky successfully transmitted tobacco mosaic disease by cell-free extracts. In addition, Sanarelli in Montevideo in 1898 had recognized filterable agents as the cause of an acute proliferative disease, myxomatosis in rabbits.

Even in 1907, when Ciuffo in Rome conducted self-inoculation experiments with cell-free extracts from human warts and subsequently developed cutaneous warts at the inoculation site, this result barely created enthusiasm. One year later, in 1908, Ellermann and Bang in Copenhagen reported the cell-free transmission of chicken leukemia. The nature of leukemia and its relationship to malignant diseases were not known in those days. Thus, these experiments were not immediately recognized as the first successful transmission of a natural malignancy. However, this changed in 1911, when Peyton Rous at the Rockefeller Institute in New York demonstrated the cell-free transmission of a solid tumor – a chicken sarcoma – which was, undoubtedly, acknowledged as a malignant neoplasm. This was a first turning point in the public recognition of studies on the infectious origin of cancers.

1.2 Frustration and Successes (1912–1950)

Unfortunately, the following years failed to provide evidence for the applicability of the Rous data to human tumors, and even the cell-free transmission of many animal tumors to the same or to different species attempted during this period commonly resulted in frustration. In fact, more than 20 years passed before any further progress was recognized.

In 1932, Richard E. Shope at the Rockefeller Institute in Princeton noted fibromatous tumors in a wild cotton-tail rabbit. Shope could readily transmit these fibromas by cell-free extracts to either domestic or cotton-tail rabbits. Interestingly, this infection caused a partial cross-immunity against rabbit myxomatosis virus. Both conditions were later identified as being caused by members of the poxvirus family.

Only one year later (in 1933), Shope also discovered a filterable agent in cotton-tail rabbit papillomas; this induced papillomas when inoculated into the scarified skin. Shope partially characterized this virus and noted its remarkable heat stability as it was able to tolerated exposure at 65 C for 30 minutes. In 1934, Rous and Beard discovered that this infection also had malignant potential; this was especially noted in domestic rabbits, where many of the initial papillomas converted into squamous cell carcinomas. This conversion also occurred sporadically in its natural host, the cotton-tail rabbit, albeit at a lower rate (Syverton and Berry, 1935). During the following

years, Rous and his coworkers continued to study this system, and investigated in particular the interaction of this virus infection with chemical carcinogens. Even after systemic application of the virus, they found a remarkable degree of synergism between infection and skin tarring or treatment of the skin with defined chemical carcinogens in carcinoma development (Rous and Kidd, 1938; Rous and Friedewald, 1944). In 1961, Ito and Evans showed that the carcinomas contained infectious papillomavirus DNA. Peyton Rous was conceptually far ahead of his time, his early studies having resulted in defining initiation as an early event in carcinogenesis, despite his not understanding the underlying mechanism. Finally, in 1966 – some 55 years after his seminal discovery in 1911 – Rous was awarded the Nobel Prize.

The 1930s were a relatively fruitful period for tumor virology: in 1936, Bittner described a "milk factor" which was transmissible from lactating mice to their offspring. The milk factor was first visualized in 1948 (Porter and Thompson), and Kinosita et al. (1953) characterized the virus in ultrathin sections by electron microscopy. The virus was later identified as a member of the retrovirus family, and its name was changed from Bittner factor or milk factor to mouse mammary tumorvirus (MMTV).

In 1938, Lucké reported a carcinoma of the kidney in the leopard frog (*Rana pipiens*), which was apparently caused by a transmissible virus. This tumor was known to be more prevalent in frogs during the cold season than in summer (McKinnel and McKinnel, 1968), and subsequently in 1956 Fawcett identified typical herpesvirus particles (now labeled as Lucké herpesvirus) in the tumors of winter frogs.

In 1907, an infectious chicken neurolymphomatosis had been recognized by the Hungarian veterinarian J. Marek, the condition subsequently being designated as Marek's disease. Some 20 years later, in 1926, Pappenheimer et al. recognized the neoplastic nature of this disease, while in 1969 Witter et al. identified the infectious agent as a member of the herpesvirus family. Thus, by the late 1960s two animal herpesviruses were being considered as etiological agents for malignant tumors in frogs and chickens.

Unfortunately, the promising studies of the 1930s were interrupted by the Second World War, and during the postwar period it took about 10 more years before any significant progress in this area re-emerged.

1.3 The Period from 1950 to 1965

Although numerous attempts had been made previously to identify an infectious etiology of at least some human tumors, the results had proved – until 1964 – to be rather disappointing. The involvement of parasites seemed true for some cancers outside of Europe and the United States, and appeared to represent an exotic curiosity. Even up to the early 1980s, most epidemiologists at best marginally considered a possible relationship between infections and cancer. Yet, the foundations for our present understanding of the specific function of tumorviruses were laid between 1950 and 1965.

In 1950 and 1951, Ludwik Gross in New York published the results of his pioneering studies on the transmission of murine leukemias following the inoculation of cell-free extracts into newborn mice. Gross had noticed that the susceptibility to cancer induction by viruses (later identified as members of the retrovirus family) depended largely on infections early in life. His studies in 1953 and 1955 resulted in the discovery of another cancer-inducing agent, later identified and described in more detail by Stewart et al. (1957) and designated polyomavirus.

In 1956 and 1957, Charlotte Friend isolated a virus which caused erythroblastosis in mice, and was able to pass it serially in weanling mice. This virus, which caused rapid enlargement of the spleen and liver and led to progressive anemia, was later identified as a member of the retrovirus family. In contrast to the earlier observations by Gross, Friend was also able to induce this proliferative condition in adult mice.

During the ensuing years, a number of additional retrovirus types were analyzed in other rodents, in chicken, cats, cattle, and even in nonhuman primates. Most frequently these infections were linked to leukemias or lymphomas in their respective hosts, and for these reasons many virologists suspected that human proliferative diseases of the hematopoietic system might also be caused by members of the same virus family.

Two other important observations were made during the early 1960s: (i) the discovery of a transforming and tumor-inducing small DNA virus, initially isolated from rhesus monkey kidney cells; and (ii) the identification of tumor-inducing properties of the widely spread human adenoviruses.

In 1960, Sweet and Hilleman described the simian vacuolating virus, labeled simian virus 40 or SV40, which was isolated from rhesus and cynomolgus monkey kidney cell culture material. One year later, Eddy et al. (1961) noted that the inoculation of rhesus monkey kidney extracts into newborn hamsters resulted after several months in invasively growing tumors. During the following year, this group identified the "oncogenic" substance as simian virus 40 (Eddy et al., 1962).

The tumors induced by SV40 failed to synthesize infectious virus, but did produce a virus-specific antigen, the Tumor- or T-antigen (Black et al., 1963 a). Similar observations were made two years later for polyomavirus-induced tumors (Habel, 1965). Subsequently, T-antigen expression was also found in the early phase of lytic infection (Pope and Rowe, 1964; Rapp et al., 1964). The availability of a virus system which would readily transform a variety of tissue culture cells without virus production, permitted the development of novel experimental approaches aimed at understanding the molecular mechanisms of cancer. The persistence of integrated SV40 DNA in transformed cells added to the interest (Sambrook et al., 1968), and within a short time studies on cell transformation by SV40 became a favorite system of a large number of tumorvirologists. The subsequent isolation of two related DNA viruses directly from humans, namely BK virus (Gardner et al., 1971) and JC virus (Padgett et al., 1971), seemed to underline further the importance of this virus group as potential carcinogens.

The other important result obtained during this period was the identification of the oncogenic properties of virus infections that were widespread among the

human population; these were the adenoviruses, which most frequently caused either respiratory or gastrointestinal symptoms upon infection. In 1962, Trentin and coworkers reported that adenovirus type 12 induced tumors when inoculated into newborn hamsters. These results were soon confirmed and extended by Huebner et al. (1962) for adenovirus type 18, by Girardi et al. (1964) for adenovirus type 7, and by Pereira et al. (1965) for adenovirus type 31. Huebner's group also demonstrated specific complement-fixing antigens in adenovirus-free hamster and rat tumors (Huebner et al., 1963).

Thus, by the mid-1960s several human pathogenic viruses had been identified which possessed oncogenic potential for newborn rodents. Although these viruses were also able to stimulate the permanent growth of tissue culture cells (immortalization), and left their footprints as T-antigens in every tumor cell, none of them transformed human cells or seemed to persist in human cancers. Nevertheless, this period stimulated a number of laboratories to search for viruses, or their footprints, in human tumors.

1.4
A First Human Tumorvirus?

In 1958, Dennis Burkitt, a British surgeon working in equatorial Africa, noted a specific childhood lymphoma that occurred only in specific geographic regions. As these regions coincided with areas of holoendemic malaria, Burkitt speculated that this tumor should have an infectious etiology, most likely vectored by an arthropod, possibly by a mosquito (1962). These initial observations by Burkitt stimulated interest among the scientific community, and consequently Pulvertaft (1964) in Western Nigeria and Epstein and Barr (1964) in Bristol, UK, began to develop tissue culture techniques for these tumors and to establish a number of lymphoblastoid lines. Epstein et al. (1964) noted herpesvirus-like particles in a small fraction of these cells, but in contrast to herpesviruses known at this time, they were unable to transfer the infection to other cell systems, embryonated chicken eggs, or to conventional laboratory animals. These authors concluded at an early stage that they had found a new member of the herpesvirus family which later, was named Epstein–Barr virus (EBV).

The subsequent development of an immunofluorescent test to detect viral antigens in virus-producing cells facilitated further studies (Henle and Henle, 1966). The availability of this test system resulted in the detection of highly elevated antibody titers against viral antigens in patients with Burkitt's lymphoma (Henle and Henle, 1966; Henle et al., 1969), and subsequently also in a second human cancer, in nasopharyngeal carcinomas (Old et al., 1966). In addition, these tests indicated that EBV must be widely spread among all human populations. In 1968, Henle's group identified EBV as the causative agent of infectious mononucleosis (Henle et al., 1968).

The first hints for an oncogenic potential of EBV originated from co-cultivation studies of lethally X-irradiated Burkitt's lymphoma tissue culture cells with umbili-

cal cord lymphocytes (Henle et al., 1967). This resulted in the regular establishment of lymphoblastoid lines of cord blood origin, and was further underlined by the discovery of persisting EBV DNA in "virus-negative" Burkitt's lymphoma cells (zur Hausen and Schulte-Holthausen, 1970), as well as in primary biopsies from Burkitt's lymphomas and nasopharyngeal cancer (zur Hausen et al., 1970). The discovery of a complement-fixing specific nuclear antigen (Pope et al., 1969; Reedman and Klein, 1973), the induction of lymphoproliferative disease after inoculation of EBV into cotton-top marmosets (Shope et al., 1973) or owl monkeys (Epstein et al., 1973), and the identification of EBV persistence in epithelial nasopharyngeal carcinoma cells (Wolf et al., 1973) added to the evidence in this early phase.

Retrospectively, it is surprising that these exciting discoveries received relatively little attention from the scientific community. This was due in part to difficulties in understanding a role for a virus in cancer induction that is persistently present in the vast majority of all human populations. The remarkable geographic restriction of the incriminated human cancers, Burkitt's lymphoma and nasopharyngeal cancer, posed an additional problem. Another reason was that problems which arose during the 1970s created an atmosphere of general disbelief for an infectious etiology of human cancers.

1.5 The Difficult 1970s

The frequent identification of retroviruses from animal leukemias and lymphomas, as well as from some epithelial tumors, resulted in intensified efforts to identify members of this virus family which also corresponded with human malignancies. A number of reports were published during these years, claiming the isolation of C-type viruses from human leukemias or finding components of these viruses in the respective tumor cells. One series of reports characterized a virus isolated from acute myelogenous leukemia (Gallagher and Gallo, 1975; Gallagher et al., 1975; Teich et al., 1975). This virus proved to be closely related to – if not identical with – woolly monkey type C virus. In another set of experiments, DNA from several patients with leukemia was found to hybridize with 70% of RNA from baboon endogenous C RNA virus (BaEV), whereas DNA from normal human tissues hybridized only with 23% of BaEV-RNA (Reitz et al., 1976; Wong-Staal et al., 1976). Based on these data, the authors claimed the horizontal transmission of a BaEV-related virus among humans. In 1977, the same group (Aulakh and Gallo, 1977) reported sequences complementary to Rauscher murine leukemia virus in some patients with leukemia, Hodgkin's disease, and multiple myeloma. These sequences were not detected in non-neoplastic spleen and kidney biopsies from a patient without neoplasia. Thus, at the time these authors suggested that at least three types of type-C viral sequences were present among the human population.

The discovery of a viral RNA-dependent DNA polymerase, reverse transcriptase (which was postulated by Howard Temin in 1964, and demonstrated by Temin and Mizutani and by Baltimore in 1970), seemed to open a new experimental approach

for the search of retroviruses in human tumors. Several reports were made on the detection of a high molecular-weight RNA associated with an RNA-instructed DNA polymerase in various human tumors, such as human leukemias (Gallo and Spiegelman, 1974), lymphomas (Spiegelman, 1975), human melanomas (Hehlmann et al., 1978) and human skin cancers (Balda et al., 1975). Unfortunately, none of these reports was later confirmed by other groups.

A third line of publications covered the possible presence of MMTV-like viruses in human breast cancer and milk. Electron microscopic investigations, as well as biochemical and biophysical analyses, suggested the presence of MMTV-like particles in human milk and malignant breast tumors (Moore 1974; Schlom et al., 1975). Particularly in the milk from women of the Parsee community in India, where there was a high incidence of breast cancer, polymerase and RNA studies provided early evidence for the existence of a MMTV-related virus in humans (Das et al., 1972). Although even controversial today, these reports still require confirmation.

It was the coincidence of these numerous reports during the 1970s (part of them presumably originating from inadvertent contaminations), and the inability of many other groups to confirm these findings that resulted in a widespread distrust and disbelief in a role of viruses in human cancers.

One other aspect added to the problems of tumor virology during the 1970s. In 1976, when Stehelin et al. reported the cellular origin of retroviral oncogenes, this had an immediate effect on tumorvirus research. Although modified cellular protooncogenes were clearly sufficient to stimulate cell growth and mediate cellular transformation, Knudson in 1971 proposed another class of genes – the tumor suppressor genes – based on his studies on retinoblastoma development. The failure of these genes is supposed to activate potential oncogenic functions within the affected cell. The identification and isolation of the retinoblastoma susceptibility gene Rb in 1986 and 1987, the demonstration of its modification in retinoblastomas and osteosarcomas (Friend et al., 1986; Lee et al., 1987), and the identification of a number of additional tumor suppressor genes paved the way for a new interpretation of cancer development. Accordingly, cancer development results from an interruption of the interplay of tumor suppressor genes and protooncogenes. This is usually mediated by mutations or loss of the suppressing alleles, or by activating mutations in oncogenes. This relatively simple and straightforward concept did not require any interaction with foreign, predominantly viral nucleic acids. In fact, it was only disturbing an otherwise clear-cut concept.

It was only consequent that, based on these considerations, a substantial number tumorvirologists turned to cell biology and the characterization of gene/gene interactions in normal and malignant cells.

1.6
The Re-Emergence of a Concept

Ten years later, the situation gradually started to change, due mainly to the contributions of three independent findings: (i) the discovery of a role of hepatitis B virus in

liver cancer; (ii) the identification of a retrovirus in a rare form of human leukemia; and (iii) the characterization of novel types of papillomaviruses causing the second most frequent cancer in females, cancer of the cervix.

The history of the hepatitis B virus and its role in hepatocellular carcinoma (HCC) is not easily unraveled. As early as the 1950s, British and French pathologists in Africa noted the frequent coincidence of hepatitis infections and hepatocellular cancer (for a review, see Szmuness, 1978). In 1956, Payet et al. suggested directly that HCC is the consequence of chronic viral hepatitis. Further, some of the early epidemiological studies stressed a role for chronic hepatitis B infections in HCC development (Prince et al., 1970; Vogel et al., 1970; Denison et al., 1971; Sankalé et al., 1971; Teres et al., 1971; Nishioka et al., 1973; Trichopoulos et al., 1975; Larouzé et al., 1976). Clear-cut evidence of this was presented a few years later when, in a prospective study conducted in government employees in Taiwan, Beasley et al. (1981) noted an increase in relative risk by a factor of 103 for HCC of hepatitis B carriers in comparison to hepatitis B-negative individuals. This clearly emphasized an important role of hepatitis B in the development of liver cancer.

Although it is still unclear by which mechanism hepatitis B virus contributes to the emergence of liver cancer, vaccination studies performed today underline the importance of this infection for hepatocellular carcinomas (this will be discussed in Chapter 5, Section 5.3).

In 1980, the isolation of a human T-lymphotropic retrovirus (HTLV-1) was reported from a cutaneous T-cell lymphoma (Poiesz et al., 1980). The virus could be propagated in T-cell growth factor-stimulated lymphocytes. The factor, interleukin-2, was previously purified and characterized by the same group (Mier and Gallo, 1980). Japanese researchers later identified the same virus (Miyoshi et al., 1981), and linked this infection to adult T-cell leukemia, which is endemic in the coastal regions of Southern Japan (Hinuma et al., 1981). These findings were rapidly reproduced, and firmly established at least one member of the retrovirus family as the causative agent of a rare form of human leukemia. Today, the link is basically proven.

Studies on papillomaviruses have a long history, in part already described for the cotton-tail papillomavirus. Early studies on the cell-free transmission of bovine warts were initiated by Magelhaes in Brazil (1920) while later, in 1951, Olson and Cook showed that the transmission of these viruses to another species, horses, resulted in the induction of sarcoids. These invasively growing but nonmetastasizing tumors are also noted in domestic horses under natural conditions. The Olson group made another striking observation, namely the induction of bladder tumors in cattle by bovine papillomavirus (BPV) infection (Olson et al., 1959). Only four years later, two additional reports by Black et al. (1963 b) and Thomas and colleagues (1963) demonstrated the transforming activity of BPV preparations for bovine fetal and murine cells.

The analysis of human papillomatous lesions and their relationship to virus infections and carcinogenesis had a much slower start. Although the infectious etiology of human warts had been clearly established based on their cell-free transmission, they were mainly regarded as a cosmetic nuisance and not thought to be of any significant medical interest.

The gradual change of this view originated from the description of a syndrome first reported by Lewandowsky and Lutz in Basel, in 1922. These authors described an hereditary condition, characterized by an extensive verrucosis, epidermodysplasia verruciformis. In these patients, at sun-exposed sites such as the forehead, the face, and the backs of the hands and arms, some papillomatous lesions converted into squamous cell carcinomas. Lutz, in 1946, and subsequently Jablonska and Millewski in 1957, proved the viral etiology of these warts in autoinoculation experiments. It was mainly the merit of Stefania Jablonska to point out a potential role of the human papillomavirus (HPV) particles seen in these warts as causal factors for the subsequent development of squamous cell cancers of the skin (Jablonska et al., 1972). Working jointly with Gérard Orth, these groups successfully demonstrated the presence of novel types of papillomaviruses, most frequently HPV 5, within epidermodysplasia verruciformis lesions and within biopsies of squamous cell carcinomas of those patients. (Orth et al., 1978, 1979).

Although HPV 5 represents the first papillomavirus infection regularly detected in cutaneous squamous cell cancers of epidermodysplasia patients, the rarity of the syndrome, the difficulties in obtaining sufficient clinical materials for extensive studies, and the absence of tissue culture lines from these carcinomas were probably the reasons for a limited interest in this condition. Only in recent years has the study of cutaneous papillomavirus infections and their relationship to nonmelanoma skin cancer in immunosuppressed and immunocompetent patients received increasing attention.

Another track of papillomavirus research resulted in the identification of specific HPV types as causative agents for cancer of the cervix, other anogenital cancers, and a subset of oropharyngeal carcinomas. These investigations began with the search for a viral etiology of cancer of the cervix, but by the late 1960s and 1970s the results of serological studies had suggested a role for human herpes simplex virus type 2 (HSV 2) in this cancer (Rawls et al., 1968; Naib et al., 1969). The present author's group was unable to confirm these findings, and sought alternative viral candidates. A number of anecdotal reports on the malignant conversion of genital warts (condylomata acuminata), scattered among the medical literature of the preceding 100 years, attracted attention. Subsequently, a possible causal role of papillomavirus infections for cervical cancer was postulated, and initial attempts were begun to characterize the viral DNA in genital warts (zur Hausen et al., 1974, 1975; zur Hausen 1976, 1977). These and other studies had the early consequence of discovering the heterogeneity of the papillomavirus family (Gissmann and zur Hausen, 1976; Orth et al., 1977; Gissmann et al., 1977), presently counting close to 106 fully sequenced genotypes (de Villiers, 1994; also Personal communication). However, the eventual isolation of HPV DNA from genital warts, labeled as HPV 6 (Gissmann and zur Hausen, 1980), and from laryngeal papillomas (HPV 11) two years later (Gissmann et al., 1982), did not yield reproducibly positive data for these viruses in cervical cancer. Yet, the use of their DNA in hybridization experiments, performed under conditions of reduced stringency, permitted the subsequent cloning of HPV 16 (Dürst et al., 1983) and HPV 18 DNA (Boshart et al., 1984), the two papillomavirus types most frequently found in cervical cancer. This allowed further ex-

periments to be conducted that would prove the role of these papillomaviruses in causing this malignancy (see Chapter 5).

The identification of three viral families with representative types that clearly cause cancers (including common carcinomas such as cancer of the cervix and liver) gradually resulted in an acceptance of infectious agents as important human carcinogens. The subsequent identification of additional infections clearly linked to other cancers further strengthened the role of infectious agents in human cancers. The hepatitis C virus was identified in 1989 (Choo et al., 1989), and initial reports on its relationship to a subset of hepatocellular carcinomas appeared in the same year (Bargiggia et al., 1989; Simonetti et al., 1989). Some earlier reports had been made, however, linking non-A, non-B hepatitis infections to liver cancer (Kiyosawa et al., 1982; Resnick et al., 1983; and others). Human herpesvirus type 8 was discovered in 1994 (Chang et al.) as being the most likely causative agent for Kaposi's sarcoma, while in 1989 (Forman et al., 1991; Nomura et al., 1991; Parsonnet et al., 1991) and 1993 (Wotherspoon et al.), *Helicobacter pylori*, as a bacterial infection, was added to the list of potential human carcinogens. Subsequently, a large number of additional HPV genotypes has been added, the pathogenic significance of which remains to be determined. The same is true for the recently discovered TT viruses; these clearly represent a new virus family, establishing probably life-long persistent infections in a high proportion of the human population.

Thus, today – more than 100 years after the first attempts to link infections to human cancer, and after more than 80 years of mainly frustrating experimentation – infections causing cancer emerge as a major factor in human carcinogenesis. This mode of research leads to new approaches towards cancer diagnosis and treatment, and – most importantly – of cancer prevention.

References

Askanazy, M. Über Infektion des Menschen mit *Distomum felineum* (*sibiricum*) in Ostpreussen und ihren Zusammenhang mit Leberkrebs. *Centr. Bakt. Orig.* 28: 491–502, **1900**.

Aulakh, G.S. and Gallo, R.C. Rauscher-leukemia-virus-related sequences in human DNA: presence in some tissues of some patients with hematopoietic neoplasias and absence in DNA from other tissues. *Proc. Natl. Acad. Sci. USA* 74: 353–357, **1977**.

Bailey, W.S. Parasites and cancer: sarcoma in dogs associated with *Spirocerca lupi*. *Ann. N.Y. Acad. Sci.* 108: 890–923, **1963**.

Balda, B.R., Hehlmann, R., Cho, J.R., and Spiegelman, S. Oncornavirus-like particles in human skin cancers. *Proc. Natl. Acad. Sci. USA* 72: 3697–3700, **1975**.

Baltimore, D. RNA-dependent DNA polymerase in virions of RNA tumour viruses. *Nature* 226: 1209–1211, **1970**.

Bargiggia, S., Piva, A., Sangiovanni, A., and Donato, F. [Primary carcinoma of the liver and hepatitis C virus in Italy. A prospective study in patients with cirrhosis] *Medicina* (*Firenze*) 9: 424–426, **1989**.

Beasley, R.P., Hwang, L.Y. Lin, C.-C., and Chien, C.-S. Hepatocellular carcinoma and hepatitis B virus. A prospective study of 22707 men in Taiwan. *Lancet* II: 1129–1133, **1981**.

Beyerinck, M.W. Über ein Contagium vivum fluidum als Ursache der Fleckenkrankheit der Tabakblätter. *Verhandl. Konink. Akad. Wetenschappen te Amsterdam* 6: 3–22, **1898**.

Bittner, J.J. Some possible effects of nursing on the mammary gland tumor incidence in mice. *Science* 84: 162, **1936**.

Black, P.H., Rowe, W.P., Turner, H.C., and Huebner, R.J. A specific complement-fixing antigen present in SV40 tumor and transformed cells. *Proc. Natl. Acad. Sci. USA* 50: 1148–1156, **1963 a**.

Black, P.H., Hartley, J.W., Rowe, W.P., and Huebner, R.J. Transformation of bovine tissue culture cells by bovine papilloma virus. *Nature* 199: 1016–1018, **1963 b**.

Boshart, M., Gissmann, L., Ikenberg, H., Kleinheinz, A., Scheurlen, W., and zur Hausen, H. A new type of papillomavirus DNA, its presence in genital cancer and in cell lines derived from genital cancer. *EMBO J*. 3: 1151–1157, **1984** .

Burkitt, D. A sarcoma involving the jaws in African children. *Br. J. Surg*. 46: 218–223, **1958**.

Burkitt, D. A children's cancer dependent on climatic factors. *Nature* 194: 232–234, **1962**.

Chang, Y., Cesarman, E., Pessin, M.S., Lee, F., Culpepper, J., Knowles, D.M., and Moore, P.S. Identification of herpesvirus-like DNA sequences in AIDS-associated Kaposi's sarcoma. *Science* 266: 1865–1869, **1994**.

Choo, Q.L., Kuo, G., Weiner, A.J., Overby, L.R., Bradley, D.W., and Houghton, M. Isolation of a cDNA clone derived from a blood-borne non-A, non-B viral hepatitis genome. *Science* 244: 359–362, **1989**.

Ciuffo, G. Innesto positive con filtrato di verruca vulgare. *Giorn. Ital. Mal. Venereol.* 48:12, **1907**.

Das, M.R., Vaidya, A.B., Sirsat, S. M., and Moore, D.H. Polymerase and RNA studies on milk virions from women of the Parsi community. *J. Natl. Cancer Inst*. 48: 1191–1196, **1972**.

Denison, E.K., Peters, R.L., and Reynolds, T.B. Familial hepatoma with hepatitis-associated antigen. *Ann. Intern. Med*. 74: 391–394, **1971**.

de Villiers, E.-M. Human pathogenic papillomaviruses: an update. *Curr. Top. Microbiol. Immunol*. 186: 1–12, **1994**.

Dürst, M., Gissmann, L., Ikenberg, H., and zur Hausen, H. A papillomavirus DNA from a cervical carcinoma and its prevalence in cancer biopsy samples from different geographic regions. *Proc. Natl. Acad. Sci. USA* 80: 3812–3815, **1983** .

Eddy, B.E., Borman, G.S., Berkeley, W.H., and Young, R.D. Tumors induced in hamsters by injection of rhesus monkey kidney cell extracts. *Proc. Soc. Exp. Biol. Med*. 107: 191–197, **1961**.

Eddy, B.E., Borman, G.S., Grubbs, G.E., and Young, R.D. Identification of the oncogenic substance in rhesus monkey kidney cell cultures as Simian Virus 40. *Virology* 17: 65–75, **1962**.

Epstein, M.A. and Barr, Y.M. Cultivation in vitro of human lymphoblasts from Burkitt's malignant lymphoma. *Lancet* 1: 252–253, **1964**.

Epstein, M.A., Achong, B.G., and Barr, Y.M. Virus particles in cultured lymphoblasts from Burkitt's lymphoma. *Lancet* 1: 702–703, **1964**.

Epstein, M.A., Hunt, R.D., and Rabin, H. Pilot experiments with EB virus in owl monkeys (*Aotus trivirgatus*) I. Reticuloproliferative disease in an inoculated animal. *Int. J. Cancer* 12: 309–318, **1973**.

Fawcett, D.W. Electron microscope observations on intracellular virus-like particles associated with the cells of the Lucke renal adenocarcinoma. *J. Biophys. Biochem. Cytol*. 2(6): 725–741, **1956**.

Forman, D., Newell, D.G., Fullerton, F., Yarnell, J.W., Stacey, A.R., Wald, N., and Sitas, F. Association between infection with *Helicobacter pylori* and risk of gastric cancer: evidence from a prospective investigation. *Br. Med. J* . 302: 1302–1305, **1991**

Friend, C. The isolation of a virus causing a malignant disease of the hematopoietic system in adult Swiss mice (Abstract). *Proc. Am. Ass. Cancer Res*. 2: 106, **1956**.

Friend, C. Cell-free transmission in adult Swiss mice of a disease having the character of a leukemia. *J. Exp. Med*. 105: 307–318, **1957**.

Friend, S. H., Bernards, R., Rogelj, S., Weinberg, R.A., Rapaport, J.M., Albert, D.M., and Dryja, T.P. A human DNA segment with properties of the gene that predisposes to retinoblastoma and osteosarcoma. *Nature* 323: 643–646, **1986**.

Gallagher, R.E. and Gallo, R.C. Type C RNA tumor virus isolated from cultured human acute myelogenous leukemia cells. *Science* 187: 350–353, **1975**.

Gallagher, R.E., Salahuddin, S. Z., Hall, W.T., McCredie, K.W., and Gallo, R.C. Growth and differentiation in culture of leukemic leukocytes from a patient with acute myelogenous leukemia and re-identification of type-C virus. *Proc. Natl. Acad. Sci. USA* 72: 4137–4141, **1975**.

Gallo, R.C. and Spiegelman, S. Letter: Reverse transcriptase in acute leukaemia. *Lancet* 1: 1117–1178, **1974**.

Gardner, S. D., Field, A.M., Coleman, D.V., and Hulme, B. New human papovavirus (B.K.) isolated from urine after renal transplantation. *Lancet* 1: 1253–1257, **1971**.

Girardi, A.J., Hilleman, M.R., and Zwickey, R.E. Tests in hamsters for oncogenic quality of ordinary viruses including adenovirus type 7. *Proc. Soc. Exp. Biol. Med.* 115: 1141–1150, **1964**.

Gissmann, L. and zur Hausen, H. Human papilloma virus DNA: physical mapping and genetic heterogeneity. *Proc. Natl. Acad. Sci. USA* 73: 1310–1313, **1976**.

Gissmann, L. and zur Hausen, H. Partial characterization of viral DNA from human genital warts (condylomata acuminata). *Int. J. Cancer* 25: 605–609, **1980**.

Gissmann, L. Pfister, H., and zur Hausen, H. Human papilloma viruses (HPV): Characterization of four different isolates. *Virology* 76: 569–580. **1977**.

Gissmann, L., Diehl, V., Schultz-Coulon, H.J., and zur Hausen, H. Molecular cloning and characterization of human papillomavirus DNA derived from a laryngeal papilloma. *J. Virol.* 44: 393–400, **1982**.

Goebel, C. Über die bei Bilharziakrankheit vorkommenden Blasentumoren mit besonderer Berücksichtigung des Carcinoms. *Zeitschr. Krebsforsch.* 3: 369–513, **1905**.

Gross, L. Susceptibility of newborn mice of an otherwise resistant strain to inoculation with leukemia. *Proc. Soc. Exp. Biol. Med.* 73: 246–248, **1950**.

Gross, L. "Spontaneous" leukemia developing in C3 H mice following inoculation in infancy, with AK-leukemic extracts. *Proc. Soc. Exp. Biol. Med.* 76: 27–32, **1951**.

Gross, L. A filterable agent, recovered from Ak-leukemic extracts, causing salivary gland carcinomas in C3 H mice. *Proc. Soc. Exp. Biol. Med.* 83: 414–421, **1953**.

Gross, L. Induction of parotid carcinomas and/or subcutaneous sarcomas in C3 H mice with normal C3 H organ extracts. *Proc. Soc. Exp. Biol. Med.* 88: 362–368, **1955**.

Habel, K. Specific complement-fixing antigens in polyoma tumors and transformed cells. *Virology* 25: 55–61, **1965**.

Hehlmann, R., Balda, B.R., and Spiegelman, S. Murine and human melanomas containing a high molecular weight RNA associated with an RNA-instructed DNA polymerase. *Int. J. Dermatol.* 17: 115–122, **1978**.

Henle, G. and Henle, W. Immunofluorescence in cells derived from Burkitt's lymphoma. *J. Bacteriol.* 91: 1248–1256, **1966**.

Henle, W., Diehl, V., Kohn, G., zur Hausen, H., and Henle, G. Herpes-type virus and chromosome marker in normal leukocytes after growth with irradiated Burkitt cells. *Science* 157: 1064–1065, **1967**.

Henle, G., Henle, W., and Diehl, V. Relation of Burkitt's tumor-associated herpes-type virus to infectious mononucleosis. *Proc. Natl. Acad. Sci. USA* 59: 94–101, **1968**.

Henle, G., Henle, W., Clifford, P., Diehl, V., Kafuko, G.W., Kirya, B.G., Klein, G., Morrow, R.H., Munube, G.M.R., Pike, P., Tukei, P.M., and Ziegler, J.L. Antibodies to Epstein-Barr virus in Burkitt's lymphoma and control groups. *J. Natl. Cancer Inst.* 43: 1147–1157, **1969**.

Hinuma, Y., Nagata, K., Hanaoka, M., Nakai, M., Matsumoto, T., Kinoshita, K.I., Shirakawa, S., and Mioshi, I. Adult T-cell leukemia: antigen in an ATL cell line and detection of antibodies to the antigen in human sera. *Proc. Natl. Acad. Sci. USA* 78: 6476–6480, **1981**.

Huebner, R.J., Rowe, W.P., and Lane, W.T. Oncogenic effects in hamsters of human adenoviruses 12 and 18. *Proc. Natl. Acad. Sci. USA* 48: 2051–2058, **1962**.

Huebner, R.J., Rowe, W.P., Turner, H.C., and Lane, W.T. Specific adenovirus complement-fixing antigens in virus-free hamster and rat tumors. *Proc. Natl. Acad. Sci. USA* 50: 379–389, **1963**.

IARC Monographs on the Evaluation of Carcinogenic Risks to Humans. Volume 61. Schistosomes, Liver flukes and *Helicobacter pylori*. IARC, Lyon, **1994**.

Ito, Y. and Evans, C.A. Induction of tumors in domestic rabbits with nucleic acid preparations from partially purified Shope papilloma virus and from extracts of the papillomas of domestic and cotton tail rabbits. *J. Exp. Med.* 114: 485–491, **1961**.

Iwanowski, D. Über die Mosaikkrankheit der Tabakpflanze. *Bull. Acad. Imp. Sci., St. Petersburg* 3: 67–70, **1894**

Jablonska, S. and Milewski, B. Zur Kenntnis der Epidermodysplasia verruciformis Lewandowsky-Lutz. *Dermatologica* 115: 1–22, **1957**.

Jablonska, S., Dabrowski, J., and Jakubowicz, K. Epidermodysplasia verruciformis as a model in studies on the role of papovaviruses in oncogenesis. *Cancer Res.* 32: 583–589, **1972**.

Kinosita, R., Erickson, J.O., Armen, DS. M., Dolch, M.E., and Ward, J.P. Electron microscope study of mouse mammary carcinoma tissue. *Exp. Cell Res.* 4: 353–4361, **1953**.

Knudson, A.G. Mutation and cancer: statistical study of retinoblastoma. *Proc. Natl. Acad. Sci. USA* 68: 820–823, **1971**.

Koch, R. Die Aetiologie der Tuberkulose. Nach einem in der Physiologischen Gesellschaft zu Berlin am 24. März 1882 gehaltenen Vortrage. *Berl. Klin. Wochenschr.* 19: 1990, **1882**.

Kiyosawa, K., Akahane, Y., Nagata, A., Koike, Y., and Furuta, S. The significance of blood transfusion in non-A, non-B chronic liver disease in Japan. *Vox Sang.* 43: 45–52, **1982**.

Larouzé, B., Saimot, G., Lustbader, E.D., London, W.T., Werner, B.G., and Payet, M., Host responses to hepatitis-B infection in patients with primary hepatic carcinomas and their families: A case-control study in Senegal, West Africa. *Lancet* 2: 534–538, **1976**.

Lee, W.-H., Bookstein, R., Hong, F., Young, L.-J., Shew, J.-Y., and Lee, E.Y. Human retinoblastoma susceptibility gene: cloning, identification and sequence. *Science* 235: 1394–1399, **1987**.

Lewandowsky, F. and Lutz, W. Ein Fall einer bisher nicht beschriebenen Hauterkrankung (Epidermodysplasia verruciformis). *Arch. Dermatol. Syph. (Berlin)* 141: 193–203, **1922**.

Lutz, W. A propos de l'epidermodysplasie verruciforme. *Dermatologica* 92, 30–43, **1946**.

Magelhaes – Verruga dos bovideos. *Brasil-Medico* 34: 430–431, **1920**.

Marek, J. Multiple Nervenentzündung (Polyneuritis) bei Hühnern. *Deutsche Tierärztl. Wochenschr.* 15: 417–421, **1907**.

McKinnell, R.G. and McKinnell, B.K. Seasonal fluctuation of frog renal adenocarcinoma. Prevalence in natural populations. *Cancer Res.* 28(3): 440–444, **1968**.

M'Fadyan, J. and Hobday, F. Note on the experimental "transmission of warts in the dog". *J. Comp. Pathol. Ther.* 11: 341–344, **1898**.

Mier, J.W. and Gallo, R.C. Purification and some characteristics of human T-cell growth factor from phytohemagglutinin stimulated lymphocyte conditioned media. *Proc. Natl. Acad. Sci. USA* 77: 6134–6138, **1980**.

Miyoshi, I., Kubonishi, I., Yoshimoto, S., Akagi, T., Ohtsuki, Y., Shiraishi, Y., Nagato, K., and Hinuma, Y. Type C virus particles in a cord T-cell line derived by co-cultivating normal human cord leukocytes and human leukaemic T cells. *Nature* 294: 770–771, **1981**.

Moore, D.H. Evidence in favor of the existence of human breast cancer virus. *Cancer Res.* 34: 2322–2329, **1974**.

Naib, Z.M., Nahmias, A.J., Josey, W.E., and Kramer, J.H. Genital herpetic infection. Association with cervical dysplasia and carcinoma. *Cancer* 23: 940–945, **1969**.

Nishioka, K., Hirayama, T., Sekine, T., Okochi, K., Mayumi, M., Jueilow, S., Chen-Hui, L., and Tong-Min, L. Australia antigen and hepatocellular carcinoma. *GANN Monogr. Cancer Res.* 14: 167–175, **1973**.

Nomura, A., Stemmermann, G.N., Chyou, P.H., Kato, I., Perez-Perez, G.I., and Blaser, M.J. *Helicobacter pylori* infection and gastric carcinoma among Japanese Americans in Hawaii. *N. Engl. J. Med.* 325: 1132–1136, **1991** .

Old, L.J., Boyse, E.A., Oettgen, H.F., De Harven, E., Geering, G., Williamson, B., and Clifford, P. Precipitating antibody in human serum to an antigen present in cultured Burkitt's lymphoma cells. *Proc. Natl. Acad. Sci. USA* 56: 1699–1705, **1966**.

Olson, C. and Cook, R.H. Cutaneous sarcoma-like lesions of the horse caused by the agent of bovine papilloma. *Proc. Soc. Exp. Biol. Med.* 77: 281–284, **1951**.

Olson, C., Pamukcu, A.M., Brobst, D.F., Kowalczyk, T., Satter, E.J., and Price, J.M. A urinary bladder tumor induced by a bovine cutaneous papilloma agent. *Cancer Res.* 19: 779–782, **1959**.

Orth, G., Favre, M., and Croissant, O. Characterization of a new type of human papillomavirus that causes skin warts. *J. Virol.* 24: 108–120, **1977**.

Orth, G., Jablonska, S., Favre, M., Jarzabek-Chorzelska, M., and Rzesa, G. Characterization of two new types of human papillomaviruses from lesions of epidermodysplasia verruciformis. *Proc. Natl. Acad. Sci. USA* 75: 1537–1541, **1978**.

Orth, G., Jablonska, S., Jarzabek-Chorzelska, M., Obalek, S., Rzesa, G., Favre, M., and Croissant, O. Characteristics of the lesions and risk of malignant conversion associated with the type of the human papillomavirus involved in epidermodysplasia verruciformis. *Cancer Res.* 39: 1074–1082, **1979**.

Padgett, B.L., Walker, D.L., Zu Rhein, G.M., Eckroade, R.J., and Dessel, B.H. Cultivation of papova-like virus from human brain with progressive multifocal leukencephalopathy. *Lancet* 1: 1257–1260, **1971**.

Pappenheimer, A.M., Dunn, L.C., and Cone, V. A study of fowl paralysis (neuro-lymphomatosis gallinarum). *Storrs Agric. Exp. Station Bull.* 143: 185–290, **1926**.

Parsonnet, J., Friedman, G.D., Vandersteen, D.P., Chang, Y., Vogelman, J.H., Orentreich, N., and Sibley, R.K. *Helicobacter pylori* infection and the risk of gastric carcinoma. *N. Engl. J. Med.* 325: 1127–1131, **1991**.

Payet, M., Camain, R., and Pene, P. Primary cancer of the liver. Critical study of 240 patients. *Rev. Int. Hepatol.* 6: 1–86, **1956**.

Pereira, M.S., Pereira, H.G., and Clarke, S. K.R. Human adenovirus type 31. A new serotype with oncogenic properties. *Lancet* 1: 21–23, **1965**.

Poiesz, B.J., Ruscetti, F.W., Gazdar, A.F., Bunn, P.A., Minna, J.D., and Gallo, R.C. Detection and isolation of type C retrovirus particles from fresh and cultured lymphocytes of a patient with cutaneous T-cell lymphoma. *Proc. Natl. Acad. Sci. USA* 77: 7415–7419, **1980**.

Pope, J.H. and Rowe, W.P. Detection of specific antigen in SV40-transformed cells by immunofluorescence. *J. Exp. Med.* 120: 121–127, **1964**.

Pope, J.H., Horne, M.K., and Wetters, E.J. Significance of a complement-fixing antigen associated with herpes-like virus and detected in the Raji cell line. *Nature* 222: 186–187, **1969**.

Porter, K.R. and Thompson, H.P. A particulate body associated with epithelial cells cultured from mammary carcinoma of mice of a milk factor strain. *J. Exp. Med.* 88: 15–24, **1948**.

Prince, A.M., Leblanc, L., Krohn, K., Masseyeff, R. and Alpert, M.E. SH antigen and chronic liver disease. *Lancet* 2: 717–718, **1970**.

Pulvertaft, R.J.V. Cytology of Burkitt's tumour (African lymphoma). *Lancet* 1: 238–240, **1964**.

Rapp, F., Kitahara, T., Butel, J.S., and Melnick, J.L. Synthesis of SV40 tumor antigen during replication of simian papovavirus (SV40). *Proc. Natl. Acad. Sci. USA* 52: 1138–1142, **1964**.

Rawls, W.E., Tompkins, W.A., Figueroa, M.E., and Melnick, J.L. Herpesvirus type 2: association with carcinoma of the cervix. *Science* 161: 1255–1256, **1968**.

Reedman, B.M. and Klein, G. Cellular localization of Epstein-Barr virus (EBV)-associated complement-fixing antigen in producer and nonproducer lymphoblastoid cell lines. *Int. J. Cancer* 11: 499–520, **1973**.

Reitz, M.S., Miller, N.R., Wong-Staal, F.. Gallagher, G.E., Gallo, R.C., and Gillespie, D.H. Primate type-C virus nucleic acid sequences (woolly monkey and baboon types) in tissues from a patient with acute myelogenous leukemia and in viruses isolated from cultured cells of the same patient. *Proc. Natl. Acad. Sci. USA* 73: 2113–2117, **1976**.

Resnick, R.H., Stone, K., and Antonioli, D. Primary hepatocellular carcinoma following non-A, non-B posttransfusion hepatitis. *Dig. Dis. Sci.* 28: 908–911, **1983**.

Rous, P. A sarcoma of the fowl transmissible by agent separable from tumor cells. *J. Exp. Med.* 13: 397–411, **1911**.