Cancer, Infection and Immunity: A Personal Homage to Jan Svoboda*

(Rous sarcoma virus / endogenous retroviruses / pseudotypes / human immunodeficiency virus / acquired immune deficiency syndrome / cancer)

R. A. WEISS

Division of Infection and Immunity, University College London, United Kingdom

Abstract. Jan Svoboda has had an extraordinary influence on my research. Following our first meeting in 1967, he encouraged me to pursue my tentative evidence for the existence of endogenous retroviruses latent in normal cells. He introduced me to the Czech scientists, Pavel Veselý and Jan Závada, with whom I collaborated fruitfully on the transformed cell phenotype and on virus pseudotypes, respectively. Through my brief training in his laboratory in Prague I gained a breadth and depth of analysis in virology, immunology and oncology that helped me subsequently to tackle problems in AIDS and AIDS-associated malignancy at the levels of both cell biology and epidemiology.

Reflecting on my 37-year friendship with Jan Svoboda, I am surprised to realize that we have never actually published a scientific paper together. Svoboda's influence was tremendous all the same. He gave me the courage to follow what were heterodox ideas by seeking firm evidence through meticulous experiments. He also introduced me to many other investigators in Czechoslovakia (as it then was), Hungary and Russia. We met in 1967 shortly before the Prague Spring at a time when contact between scientists in Western Europe and those in countries under Soviet hegemony was not warmly promoted but was cautiously allowed to proceed. A small organization called the European Tumour Virus Group held symposia alternatively in Eastern bloc and Western bloc countries and as a young, inexperienced researcher, I attended the April 1967 meeting in Sorrento, Italy. Jan Svoboda had been permitted to spend a year in England working at the Mill Hill Laboratories of the Imperial Cancer Research Fund (ICRF) directed by Bob Harris, so we extended our acquaintance.

A common theme between the Institute of Experimental Biology and Genetics of the Czechoslovak Academy of Sciences in Prague (now the Institute of Molecular Genetics) and the ICRF Mill Hill Laboratories was the study of Rous sarcoma virus (RSV) as a model for oncogenesis and tumour immunity – in turkeys at Mill Hill and in mammalian cells in Prague. A feature of the Prague strain of RSV, in common with the Carr-Zilber strain from Moscow, the Schmidt-Ruppin strain from Zurich and the B77 strain from Bratislava, was that they were competent for both viral replication and cell transformation. In contrast, the Bryan high-titre strain that was studied in the USA lacked an *env* gene and therefore needed a 'helper' avian leukosis virus to replicate through complementation. In those days we had no gene map, but in modern understanding, these virus strains have the following genetic organization:

Prague RSV: 5' LTR-*gag-pol-env-src*-LTR 3'
Bryan RSV: 5' LTR-*gag-pol-src*-LTR 3'
Helper ALV: 5' LTR-*gag-pol-env*-LTR 3'

Although the Prague strain of RSV possessed all the genes necessary for viral replication, mammalian cells were not permissive for replication. Svoboda's seminal contribution to our understanding of RSV in the 1960s was to demonstrate rescue of replicating virus by inoculation of the rat XC cell line into chickens (Svoboda, 1961, 1962; Svoboda et al., 1963) and by mixing RSV-transformed mammalian cells with permissive chicken embryo fibroblasts (Svoboda, 1964). When he induced cell fusion between these cells using inactivated Sendai virus particles to form heterokaryons, rescue of RSV was greatly increased (Svoboda et al., 1967; Svoboda and Dourmashkin, 1969). His findings further showed that the RSV genome was persistent in mammalian transformed cells through 1000s of mitotic divisions in the apparent absence of viral replication. The persistence and rescue of DNA tumour viruses such as polyoma, SV40 and adenovirus behaved in a similar way in non-permissive, transformed cells derived from host species not naturally infected by the viruses (Gerber, 1966; Watkins and Dulbecco, 1967). Svoboda's initial observations led him to compare RSV-transformed rat

Received May 10, 2004.

The author's work is supported by Cancer Research UK and the Medical Research Council.

Corresponding author: Robin A. Weiss, Division of Infection and Immunity, University College London, 46 Cleveland Street, London W1T 4JF, United Kingdom. Fax: +44 (0)20 7679 9555; e-mail: r.weiss@ucl.ac.uk.

*Dedicated to Professor Jan Svoboda on the occasion of his 70th birthday.

Abbreviations: AIDS – acquired immune deficiency syndrome, ALV – avian leukosis virus, HIV – human immunodeficiency virus, KSHV – Kaposi's sarcoma associated herpesvirus, RSV – Rous sarcoma virus.

cells to the lysogenic condition of bacteriophage lambda (Svoboda, 1964). Thus he independently postulated a theory of RSV persistence similar to Howard Temin's DNA provirus hypothesis (Temin, 1964), but their ideas were not taken seriously by most virologists until after the discovery of reverse transcriptase by Baltimore (1970) and by Temin and Mizutani (1970). The exchange of correspondence in the 1960s between Svoboda and Temin makes interesting reading on the development of the concept (Svoboda, 2003).

At the Sorrento meeting in 1967, while still pursuing doctoral studies at University College London, I reported a curious finding, namely, that certain types of Brown Leghorn chicken embryo cells obtained from ICRF Mill Hill allowed the release of infectious Bryan strain RSV in the absence of a replicating helper virus and with a distinct host range (Weiss, 1967, 1969). At the same meeting, two other young scientists also presented preliminary data on what were later to be called endogenous retroviruses. Jim Payne described the dominant mendelian inheritance of a Gag-related antigen in chickens. Complement fixation of Gag was being used as an immunoassay to screen embryos for ALV infection to attempt to establish leukosis-free flocks; what was thought to be an immunological 'cross-reaction' with a host protein (Dougherty and Di Stefano, 1966) turned out to be an important discovery (Payne and Chubb, 1968). Peter Bentvelzen reported the mendelian inheritance of susceptibility to mammary tumours of GR mice, in contrast to the milk transmission of virus in C3H and other mouse strains; this tentative evidence for a heritable murine mammary tumour virus was later confirmed and called a germinal provirus (Bentvelzen, 1971). These exciting findings led us to postulate and demonstrate the presence of endogenous retroviruses as integrated mendelian proviruses in the host chromosomes (Payne and Chubb, 1968; Weiss, 1969; Weiss and Payne, 1971; Varmus et al., 1972). Before the discovery of reverse transcriptase our findings tended to be ridiculed, but both Svoboda and Temin warmly encouraged us to pursue our work showing that retroviruses can be transmitted either as infectious agents or as

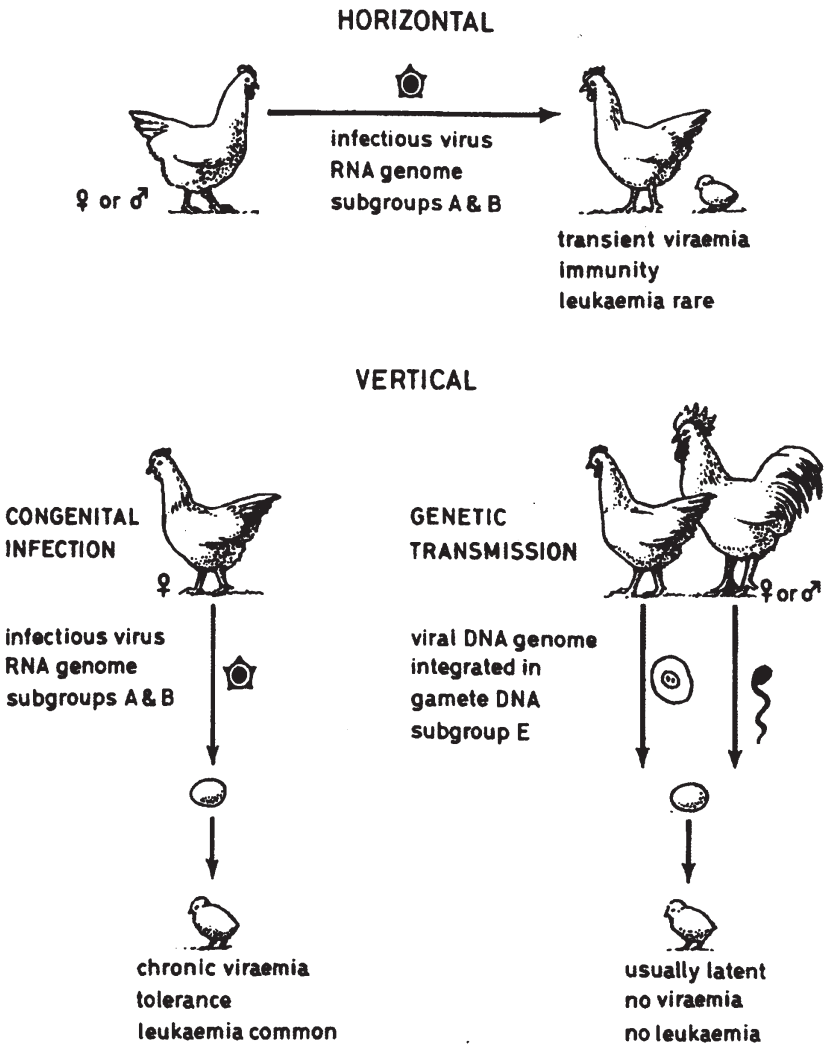

Fig. 1. Transmission of avian leukosis viruses in chickens (Weiss et al., 1985b)

mendelian genomes (Fig. 1). Today, endogenous retroviruses are accepted as text-book facts; the sequencing of the human genome revealed that some 8% of human DNA represents 'fossil' proviruses (Li et al., 2001).

During the fall of 1967, Svoboda introduced me to a young colleague, Pavel Veselý, for whom he had managed to arrange a short visit to Mill Hill. Veselý shared my interest in morphological transformation and cell locomotion of RSV-transformed cells and we engaged in joint research on time-lapse cinematography on the loss of contact inhibition. I was a student of Michael Abercrombie, who had discovered contact inhibition (Abercrombie and Heaysman, 1954), and its analysis in RSV transformation was the main thrust of my doctoral thesis. Following Veselý's return to Prague, I was invited to visit Czechoslovakia. Veselý is a keen mountaineer and I remember spending Sylvester night and the New Year weekend in deep snow in the Krkonoše mountains with Veselý and the Director of the Institute, Milan Hašek. Hašek was a brilliant immunologist, who was friendly with my former mentor, Peter Medawar, for the two had independently discovered the phenomenon of immunological tolerance.

Thus, the Prague Spring started brightly for scientific interactions between Veselý in Svoboda's laboratory, and my research in the Anatomy & Embryology Department at UCL. During the spring of 1968, Veselý came to UCL to join me in analysing Rous-transformed cells by time-lapse microcinematography (Veselý and Weiss, 1970, 1973). With such fruitful research going on, and with the encouragement of warmer Anglo-Czech relations in the climate of the Prague Spring, Hašek invited me to spend six months at his Institute. I enthusiastically accepted and a starting date was fixed for 1 September 1968. But when the Warsaw Pact tanks rolled into Czechoslovakia on the night of 20-21 August, I was in a quandary what to do. The answer arrived three days later in the form of a telegram (there was no fax or e-mail in those days) from Prague:

PLEASE POSTPONE VISIT STOP UNINVITED GUESTS ARRIVED FIRST STOP HAŠEK.

I was determined not to allow the Cold War to interfere with international co-operation in science, and managed to rearrange the 'postponed' visit to start in the summer of 1969. Hašek even sent me as his Institute's official delegate to a conference in Moscow and the train was due to leave Prague for Moscow via Slovakia and Ukraine on the night of 20 August. Fearing some sort of uprising on the first anniversary of the Soviet invasion, all the trams and buses were cancelled and tanks were placed on each bridge across the Vltava. How was I to reach the station and board the Moscow train heavily guarded by Russian troops when I spoke no Czech or Russian? It was Svoboda who came to the apartment where we were living with the Veselý's (reassuring my wife left there with our 3-year and 1-year old children that I would come to no harm), and walked me by a circuitous route to the main station. Thus I was able to present our time-lapse movies of Rous-transformed cells to the international conference in Moscow. Svoboda and Veselý had also given me names of Russian colleagues so that I became acquainted with eminent Russian virologists and cell biologists: V. Shevliagin and V. M. Zhdanov at the Ivanovsky Institute of Virology, Yuri Vasiliev, G. Svet-Moldavsky at the Institute of Oncology and a younger scientist, Tolya Altstein at the Gamaleya Institute.

In September 1969, the much smaller meeting of the European Tumour Virus Group was held in Smolenice Castle in Slovakia, hosted by the Cancer Research Institute in Bratislava. This also proved to be an eye-opener for me, meeting for the first time future research leaders such as Čestmír Altaner (who studied with Howard Temin), Marta Grofová (Mika Popovič's mentor, who baked the most delicious apple strudel I have ever tasted), Dušan Šimkovič and Jan Závada. Veselý and I also attended an excellent small workshop on microcinematography in Hradec Králové to present our studies on Rous-transformed cells (Veselý and Weiss, 1970).

In 1970, Veselý returned to UCL to continue our studies (Veselý and Weiss, 1973; Weiss et al., 1973b). We started working with Alan Boyde, a colleague who was the first to adapt scanning electron microscopy (SEM), previously used in metallurgy, to biological hard tissues such as bone and teeth. Boyde wanted to explore whether carbon and gold evaporation onto cryo-dessicated cells would permit SEM images, and we provided him with cultured tumour cells and Rous-transformed cells (Boyde et al., 1972; Veselý and Weiss, 1973). It was only later that we realized how pioneering our work was in depicting for the first time the SEM morphology of tumour cells (Fig. 2). Veselý's partnership with Boyde continues to this day (Veselý et al., 2003).

Having successfully cut my teeth as a post-doctoral scientist in Czechoslovakia, the following year I went to Malaysia by way of my wife's family in Singapore. I lived for one month with aborigine tribesman to trap wild red jungle fowl (*Gallus gallus*), the ancestor of the domestic fowl. The purpose of the project was to determine whether red jungle fowl also possessed the endogenous retroviruses found in chickens (Weiss and Biggs, 1972). Several years later, we were able to show that the ALV genome became endogenous as a mendelian provirus after the separation of the genus *Gallus* into its 4 extant species, but before the domestication of chickens (Frisby et al., 1979). In October 1970 I set off for Seattle, USA to work with Peter Vogt. I was fortunate to be awarded an Eleanor Roosevelt Fellowship of the International Union against Cancer, thanks to Svoboda providing a most supportive reference.

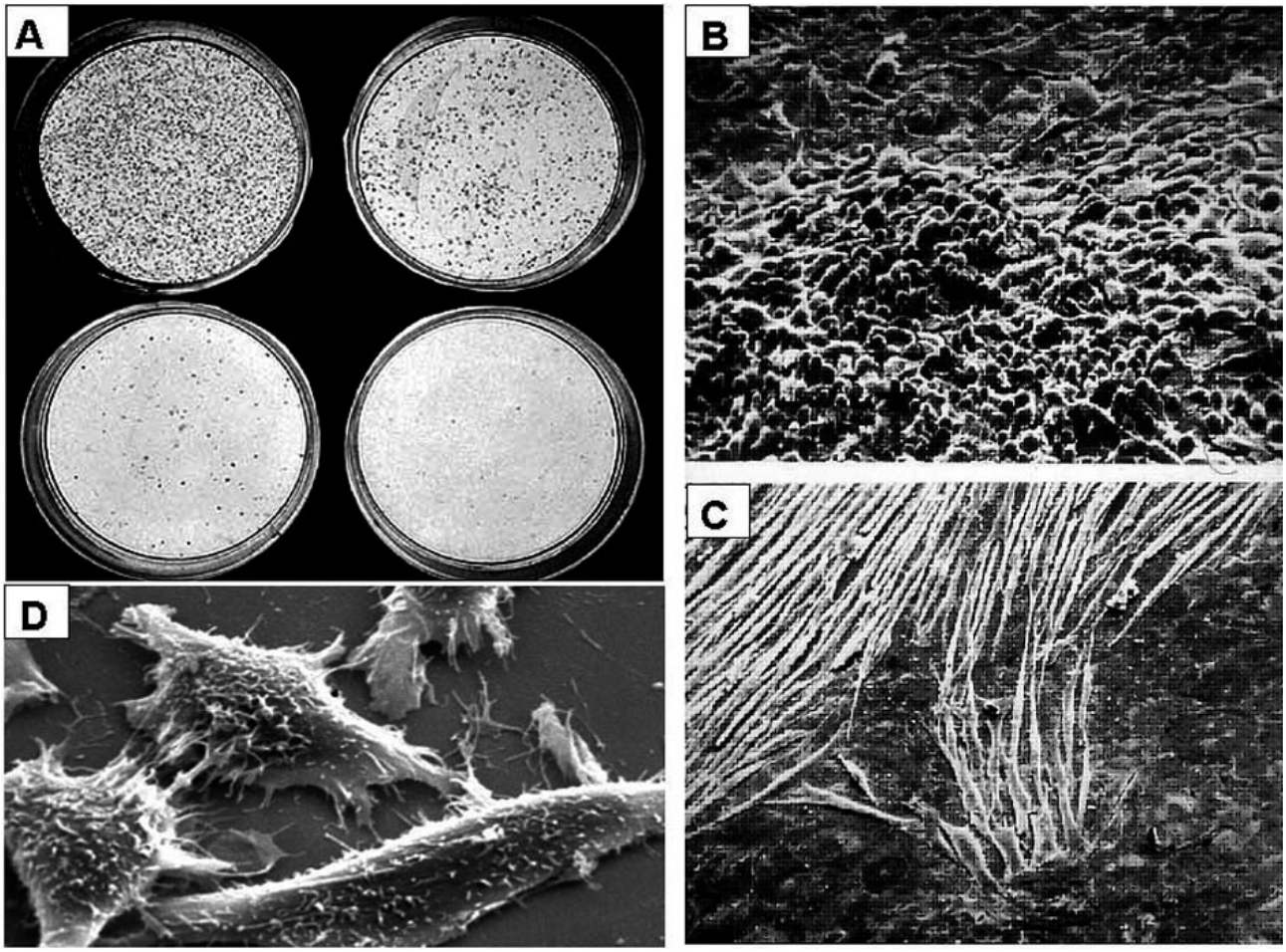

Fig. 2. Morphology of cells transformed by Rous sarcoma virus (Boyde et al., 1972; Weiss, Veselý and Boyde, unpublished)
A. Focus assay of RSV (Temin and Rubin, 1958) on quail embryo fibroblasts. Serial 10-fold dilutions in each well show the number of transformed foci as dark dots.
B. Low-power scanning electron micrograph showing a focus of transformed cells at the bottom of the panel in which the cells have lost density dependence and contact inhibition
C. Low-power scanning electron micrograph showing the fusiform variant focus of transformed cells where the transformed cells align with each other but migrate over the monolayer of normal cells
D. Scanning electron micrograph of Svoboda's RSV-transformed rat XC cells

I found the contrast between research attitudes in the USA and in the Eastern bloc illuminating. The technical and mental boldness of the best American laboratories was exhilarating; yet in some ways all the hard experimental work one could accomplish in the USA allowed a certain intellectual laziness in comparison to months of painstaking planning to perform one costly experiment in a less resource-full environment. So I came to admire the intellectual rigour and theoretical thinking that underlay the best science in Eastern Europe, while recognizing that over recent decades the West has made the major breakthroughs thanks to its economic superiority and innovative technology. Now that the Czech Republic and neighbouring countries have become integrated into the European Union, we can look forward to political stability in Europe. Moreover, new research budgets and co-operative exchange should greatly benefit research in the countries new to the EU. But I do hope that the scholars maintain their intellectual independence, as they strived to do in harsher times. The EU 6th Framework mentality for research emanating from the unelected 'nomenklatura' in Brussels often strikes me as redolent of an F1 hybrid between František Kafka's Castle and a Soviet 5 Year Plan, rather than a scheme to encourage entrepreneurial, innovative scientific thinking!

My two years with Peter Vogt converted me from a dilettante into a serious scientist. Vogt himself has been a true pioneer in tumour virology, and we developed investigations of endogenous retroviruses and retroviral recombination together (Weiss et al., 1971; Weiss et al., 1973a). During that time, I collaborated with Harold Varmus and Mike Bishop to add molecular biology methods to virus genetics in investigating endogenous retroviruses (Varmus et al., 1972). In America, I learned that it was not sufficient to be clever; one aimed to be first!

In 1971, on one of his last visits to Western Europe permitted for many years, Svoboda came to Amsterdam, for a conference honouring Otto Mühlbock, which was also attended by the present and future Nobel Laureates André Lwoff, Howard Temin and George Snell. I returned to Prague from Amsterdam with Svoboda; at the airport he was nearly arrested for insubordination when he interjected with the immigration officer who was being too officious over my visa. Back in England in 1972, I joined the ICRF Laboratories at Lincoln's Inn Fields (now the London Institute of Cancer Research UK). We proposed a formal bilateral agreement of co-operation between the ICRF and the Institute of Molecular Genetics, which was supported by the Directors, Sir Michael Stoker and Josef Říman. This agreement continued long beyond my time at ICRF. It helped Czech scientists such as Vlasta Sovová, Pavel Veselý and Jitka Forstová to make regular visits to work in London, and it helped ICRF tumour virologists – Clive Dickson, Beverly Griffin, Gordon Peters and John Wyke – to visit Prague. There was an unofficial system whereby we brought laboratory reagents available in Western countries to Prague and returned with useful antibodies and cell lines (as well as Pilsner beer and Supraphon discs). The contacts continue to this day, where Daniel Zicha is the head of microscopy at the London Institute.

My most rewarding interaction was with Jan Závada, then at the Institute of Virology in Bratislava. I adopted the vesicular stomatitis virus (VSV) envelope pseudotype system invented by Závada (1972) in order to probe the expression of endogenous retroviruses and retrovirus receptors (Love and Weiss, 1974; Boettiger et al., 1975; Teich et al., 1977). We also made reverse pseudotypes, RSV and murine leukaemia virus particles bearing VSV glycoproteins that were the precursors to today's retroviral vectors for gene transfer (Weiss et al., 1977). My research programme gained immeasurably from Závada's ideas, enthusiasm, and his generous provision of VSV thermolabile mutants and high-titre sheep antiserum. When Závada and his wife Zuzana Závadová came to ICRF for six months, we were able to tackle the cellular tropism of murine mammary tumour virus and primate retroviruses through exploitation of pseudotypes (Schnitzer et al., 1977; Závada et al., 1977) and to develop novel pseudotypes and virions bearing phenotypically mixed envelopes (Závadová et al., 1977). It was fitting many years later, after Svoboda was finally made Director of his Institute following the collapse of the communist regime, that he invited the Závada's to move to Prague when newly independent Slovakia became unfriendly to its non-Slovak minorities.

It is curious to look back and consider that almost all the tumour virologists I knew at the ICRF, in Prague and in the USA sooner or later abandoned viruses and became molecular biologists investigating oncogenes and cell cycle control. With the opening up of DNA cloning and other techniques to cell biology, viruses no longer remained the essential keys to unlock the inner workings of cancer cells. Today's generation of younger cancer researchers do not always appreciate how many of our concepts came from tumour virology – that oncogenes, such as *src*, *myc*, *ras*, *jun* and *fos*, were first discovered in RNA tumour viruses, and that tumour suppressor genes such as p53 and Rb first came to light as cellular factors put out of action by the early gene products of DNA tumour viruses. Moreover, the Central and Eastern European school of tumour virology provided several of our most important oncogenic retroviruses. For example, the *src* gene was first delineated in the non-defective RSV strains, Schmidt-Ruppin, Carr-Zilber, Prague and Bratislava (B77); the *myc* gene was captured by the myelocytoma virus first isolated in Bulgaria. The history of these pioneering findings is recorded in our textbook (Weiss et al., 1985b). Svoboda well understood the significance of these discoveries and promoted as much co-operation as was possible under difficult personal circumstances.

On moving to the Institute of Cancer Research as Director in 1980, I recruited Chris Marshall and Alan Hall to the Chester Beatty Laboratories of the Institute to search for human oncogenes via DNA transfection (Marshall et al., 1982; Hall et al., 1983). They were so successful in this endeavour that I soon felt redundant, and following Svoboda's example with talented young scientists, I relinquished the human oncogene field to them. However, it was in 1980 that the first human retroviral pathogen was discovered (Poiesz et al., 1980), so I soon reverted to retrovirology but with an interest in human infection. Both the ICRF and the Chester Beatty Laboratories had good links with Hungarian oncologists and virologists, and a string of young Hungarian scientists passed through my laboratory – Eva Gönzöl, Alan Pinter, Josef Timar and Karoly Nagy. The latter two took up the challenge of human T-cell leukaemia virus type 1 (HTLV-1). Nagy adapted a cell fusion or syncytial assay, first used by the Czech virologist Václav Klement, a former student of Svoboda. After moving to Walter Rowe's laboratory at NIH, he devised a cell fusion assay for MLV titration based on Svoboda's RSV-transformed rat cell line, XC (Klement et al., 1969). We showed that HTLV-infected cells also formed syncytia with XC cells (Nagy et al., 1983) (Fig. 3). This heterokaryon formation could be inhibited with antiserum from HTLV-infected patients. Combined with preparing Závada-type VSV pseudotypes bearing HTLV envelope antigens, we developed a precise and quantitative serology for HTLV-1, showing that the Japanese and West Indian isolates represented a single neutralizing serotype, distinct from HTLV-2 (Clapham et al., 1984).

When HIV was discovered, first at the Institut Pasteur (Barré-Sinoussi et al., 1983) and by Mika Popovič soon after (Popovič et al., 1984), we were poised to exploit exactly the same techniques that we had used for HTLV-1

but to apply them to the burgeoning epidemic of AIDS. We established pseudotype and syncytium assays for HIV to demonstrate that CD4 is the binding receptor on the surface of T-helper cells for HIV (Dalgleish et al., 1984), and predicted that a second factor (now known to be chemokine co-receptors) was required (Maddon et al., 1986). We showed that HIV-infected patients produce high-titre envelope antibodies measured by ELISA or immunofluorescence, but with extraordinarily low neutralizing activity measured by pseudotype and infectivity assays, to the detriment of vaccine development (Weiss et al., 1985a). Thus the techniques in tumour virology that I had acquired from Czech tumour virologists were put to good use in research on AIDS. Twenty years on, we are similarly using retroviral pseudotypes bearing SARS S glycoprotein to titrate neutralizing antibodies to the SARS coronavirus.

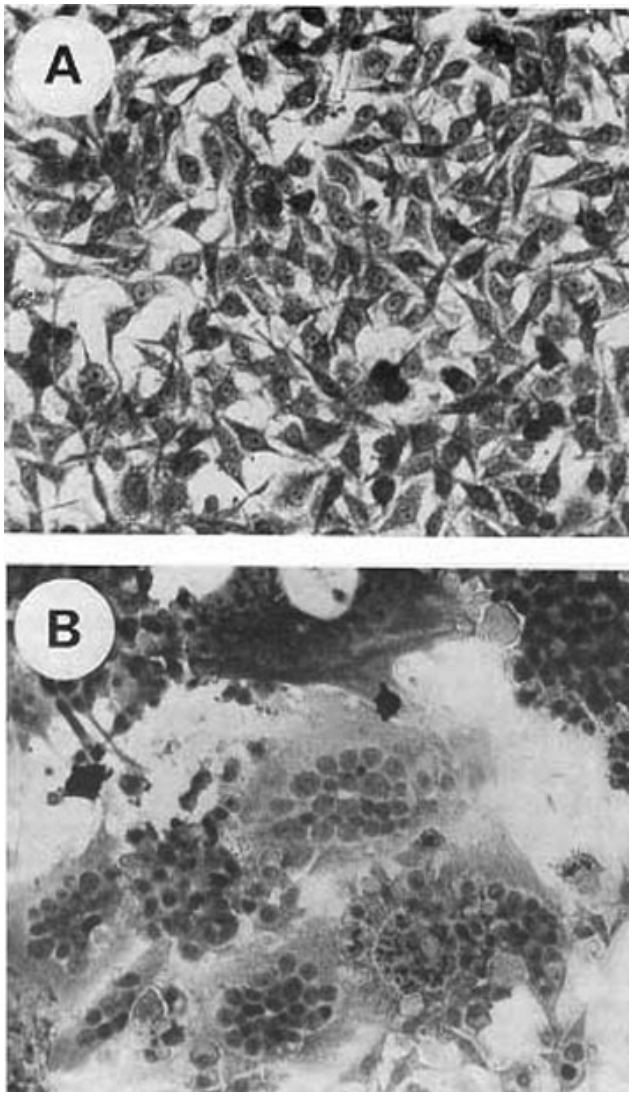

Fig. 3. Syncytia induced by HTLV-1.
A. Control RSV-transformed rat XC cells.
B. XC cells 20 hours after exposure to HTLV-1-releasing cells.

One of the strengths of the Czech school of tumour virology so ably led by Svoboda was the ease with which investigators switched between virology, oncology, immunology and genetics. I have tried to emulate this versatility in my more recent investigations of virology in immunocompromized hosts. The last time I gave a seminar at the Institute of Molecular Genetics in 1997, I spoke of my laboratory's interest in the potential infection hazards of xenotransplantation and the ability of porcine endogenous retroviruses to infect human cells (Le Tissier et al., 1997; Patience et al., 1997) via receptors that we have only recently identified (Ericsson et al., 2003). On the same visit, it was my wife who commented that Svoboda appeared to be as knowledgeable about art as science, when he took us to an exhibition of Secessionist painters in Prague. The next day Veselý kindly drove us all the way to Boskovice in Moravia to see where my grandfather spent his childhood and we encountered the local historian, Jan Branský, a retired medical doctor. Back in the Institute, the Závada's told me the fascinating story of carbonic anhydrases in malignancy (Závada et al., 2000).

In my recent research on AIDS-related malignancies, I also frequently recall my discussions with Svoboda when we were both much younger. Svoboda had started to study Rous-induced tumours in mammals as a means to understanding tumour immunity. Ever since 1908, when Paul Ehrlich first proposed the immune surveillance hypothesis of cancer control, it has been powerfully argued (especially by F. McFarlane Burnet) that without cell-mediated immunity, cancers would occur much more frequently. However, analysis of immunocompromized hosts, whether they be knock-out mice, immunosuppressed transplant patients or those infected with HIV, indicates that the common cancers are not increased. Only malignancies of the disrupted immune system itself, or the tumours caused by oncogenic human viruses appear to occur more frequently in AIDS and transplant patients, particularly B-cell lymphomas, Kaposi's sarcoma and cervical carcinoma (Boshoff and Weiss, 2002). After the Kaposi's sarcoma herpesvirus (KSHV or HHV-8) was discovered (Chang et al., 1994), we showed that it was present in the tumour cells (Boshoff et al., 1995). The switch between latency and active replication in KSHV is more complex than in RSV-transformed rat cells, and viral transforming genes are expressed both in latent and productive phases (Boshoff et al., 1997; Kellam et al., 1997). Our epidemiological study of cancer in South Africa showed that KSHV and HIV act synergistically in the emergence of Kaposi's sarcoma (Sitas et al., 1999).

We have a better understanding now of the multifactorial process of oncogenesis, and realize that a cancer cell lineage must accumulate mutations affecting oncogene activation, deletion of tumour suppressor genes, anti-apoptotic signals and defective DNA repair. That

accomplished, then a weakened immune system may also predispose to the emergence of cancer, especially if the tumor cells were previously suppressed by the expression of non-self antigens encoded by viruses. These viruses play their role in interacting with the host in specific lineages and at specific stages of development. We have recently studied this in the B-cell lineage by gene expression profiling using DNA arrays to analyse the difference between cells targeted by Epstein-Barr virus and KSHV (Jenner et al., 2003).

In his 70th year, Svoboda can take some satisfaction that species-specific host restriction of retrovirus replication is back in vogue four decades after he demonstrated rescue of RSV from mammalian cells by fusion with chicken cells permissive for viral replication. In this case it is HIV and the related simian immunodeficiency virus (SIV) that are under scrutiny. One example is innate intracellular immunity to incoming retroviruses by the APOBECG3 cytidine deaminase (Sheehy et al., 2002, 2003). This discovery explains why the complex lentiviruses require Vif as a viral infectivity factor.

Another restriction operates on Gag proteins rather like Svoboda's original studies of non-permissive RSV-transformed rat cells. The host restriction factor known as Ref-1 in human cells, and Lv-1 in simian cells, acts on Gag with cellular cyclophilin A (Towers et al., 2003). This restriction helps to explain why HIV does not propagate in many species of simian cells, and why most SIV strains will not replicate efficiently in human cells. Retroviral zoonoses might well occur more frequently if such host-specific restriction factors did not operate. The recent cloning of the gene encoding Lv-1 as TRIM-5 (Stremlau et al., 2004) opens new avenues to determining the intimate pathways of host-virus interaction governing pathogenesis. I am pleased to say that the work in our laboratory on host restriction of HIV, directed by my colleague Greg Towers, includes a graduate student, Zuzana Kečkesova from the Institute of Molecular Genetics in Prague (Kečkesova et al., 2004). Long may the tradition of exchanging young scientists continue, initiated in the 1960s by Jan Svoboda.

Acknowledgements

I am grateful to MUDr. Pavel Veselý for help on the accuracy of the manuscript.

References

Abercrombie, M., Heaysman, J. E. (1954) Observations on the social behaviour of cells in tissue culture. II. Monolayering of fibroblasts. *Exp. Cell Res.* **6,** 293-306.

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

Barré-Sinoussi, F., Chermann, J. C., Rey, F., Nugeyre, M. T., Chamaret, S., Gruest, J., Dauguet, C., Axler Blin, C., Vezinet Brun, F., Rouzioux, C., Rozenbaum, W., Montagnier, L. (1983) Isolation of a T-lymphotropic retrovirus from a patient at risk for acquired immune deficiency syndrome (AIDS). *Science* **220,** 868-871.

Bentvelzen, P. (1971) Hereditary infections with mammary tumor viruses in mice. In: *RNA Viruses and Host Genome in Oncogenesis,* eds. Emmelot, P., Bentvelzen, P., pp. 309-337, North Holland Publishing Company, Amsterdam.

Boettiger, D., Love, D. N., Weiss, R. A. (1975) Virus envelope markers in mammalian tropism of avian RNA tumor viruses. *J. Virol.* **15,** 108-114.

Boshoff, C., Weiss, R. A. (2002) AIDS-related malignancies. *Nat. Rev. Cancer* **2,** 373-382.

Boshoff, C., Schulz, T. F., Kennedy, M. M., Graham, A. K., Fisher, C., Thomas, A., McGee, J. O., Weiss, R. A., O'Leary, J. J. (1995) Kaposi's sarcoma-associated herpesvirus infects endothelial and spindle cells. *Nat. Med.* **1,** 1274-1278.

Boshoff, C., Endo, Y., Collins, P. D., Takeuchi, Y., Reeves, J. D., Schweickart, V. L., Siani, M. A., Sasaki, T., Williams, T. J., Gray, P. W., Moore, P. S., Chang, Y., Weiss, R. A. (1997) Angiogenic and HIV-inhibitory functions of KSHV-encoded chemokines. *Science* **278,** 290-294.

Boyde, A., Weiss, R. A., Veselý, P. (1972) Scanning electron microscopy of cells in culture. *Exp. Cell Res.* **71,** 313-324.

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

Clapham, P., Nagy, K., Weiss, R. A. (1984) Pseudotypes of human T-cell leukemia virus types 1 and 2: neutralization by patients' sera. *Proc. Natl. Acad. Sci. USA* **81,** 2886-2889.

Dalgleish, A. G., Beverley, P. C., Clapham, P. R., Crawford, D. H., Greaves, M. F., Weiss, R. A. (1984) The CD4 (T4) antigen is an essential component of the receptor for the AIDS retrovirus. *Nature* **312,** 763-767.

Dougherty, R. M., Di Stefano, H. S. (1966) Lack of relationship between infection with avian leukosis virus and the presence of COFAL antigen in chick embryos. *Virology* **29,** 586-595.

Ericsson, T. A., Takeuchi, Y., Templin, C., Quinn, G., Farhadian, S. F., Wood, J. C., Oldmixon, B. A., Suling, K. M., Ishii, J. K., Kitagawa, Y., Miyazawa, T., Salomon, D. R., Weiss, R. A., Patience, C. (2003) Identification of receptors for pig endogenous retrovirus. *Proc. Natl. Acad. Sci. USA* **100,** 6759-6764.

Frisby, D. P., Weiss, R. A., Roussel, M., Stehelin, D. (1979) The distribution of endogenous chicken retrovirus sequences in the DNA of galliform birds does not coincide with avian phylogenetic relationships. *Cell* **17,** 623-634.

Gerber, P. (1966) Studies on the transfer of subviral infectivity from SV40-induced hamster tumor cells to indicator cells. *Virology* **28,** 501-509.

Hall, A., Marshall, C. J., Spurr, N. K., Weiss, R. A. (1983) Identification of transforming gene in two human sarcoma cell lines as a new member of the ras gene family located on chromosome 1. *Nature* **303,** 396-400.

Jenner, R. G., Maillard, K., Cattini, N., Weiss, R. A., Boshoff, C., Wooster, R., Kellam, P. (2003) Kaposi's sarcoma-associated herpesvirus-infected primary effusion lymphoma has a plasma cell gene expression profile. *Proc. Natl. Acad. Sci. USA* **100,** 10399-10404.

Kečkesova, Z., Ylinen, L. M. A., Towers, G. J. (2004) The human and African green monkey TRIM5alpha genes encode Ref1 and Lv1 retroviral restriction factor activities. *Proc. Natl. Acad. Sci. USA*, in press.

Kellam, P., Boshoff, C., Whitby, D., Matthews, S., Weiss, R. A., Talbot, S. J. (1997) Identification of a major latent

nuclear antigen, LNA-1, in the human herpesvirus 8 genome. *J. Hum. Virol.* **1,** 19-29.

Klement, V., Rowe, W. P., Hartley, J. W., Pugh, W. E. (1969) Mixed culture cytopathogenicity: a new test for growth of murine leukemia viruses in tissue culture. *Proc. Natl. Acad. Sci. USA* **63,** 753-758.

Le Tissier, P., Stoye, J. P., Takeuchi, Y., Patience, C., Weiss, R. A. (1997) Two sets of human-tropic pig retrovirus. *Nature* **389,** 681-682.

Li, W-H., Gu, Z., Wang, H., Nekrutenko, A. (2001) Evolutionary analyses of the human genome. *Nature* **409,** 847-849.

Love, D. N., Weiss, R. A. (1974) Pseudotypes of vesicular stomatitis virus determined by exogenous and endogenous avian RNA tumor viruses. *Virology* **57,** 271-278.

Maddon, P. J., Dalgleish, A. G., McDougal, J. S., Clapham, P. R., Weiss, R. A., Axel, R. (1986) The T4 gene encodes the AIDS virus receptor and is expressed in the immune system and the brain. *Cell* **47,** 333-348.

Marshall, C. J., Hall, A., Weiss, R. A. (1982) A transforming gene present in human sarcoma cell lines. *Nature* **299,** 171-173.

Nagy, K., Clapham, P., Cheingsong Popov, R., Weiss, R. A. (1983) Human T-cell leukemia virus type I: induction of syncytia and inhibition by patients' sera. *Int. J. Cancer* **32,** 321-328.

Patience, C., Takeuchi, Y., Weiss, R. A. (1997) Infection of human cells by an endogenous retrovirus of pigs. *Nat. Med.* **3,** 282-286.

Payne, L. N., Chubb, R. C. (1968) Studies on the nature and genetic control of an antigen in normal chick embryos which reacts in the COFAL test. *J. Gen. Virol.* **3,** 379-391.

Poiesz, B. J., Ruscetti, F. W., Gazdar, A. F., Bunn, P. A., Minna, J. D., Gallo, R. C. (1980) 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.

Popovič, M., Sarngadharan, M. G., Read, E., Gallo, R. C. (1984) Detection, isolation, and continuous production of cytopathic retroviruses (HTLV-III) from patients with AIDS and pre-AIDS. *Science* **224,** 497-500.

Schnitzer, T. J., Weiss, R. A., Závada, J. (1977) Pseudotypes of vesicular stomatitis virus with the envelope properties of mammalian and primate retroviruses. *J. Virol.* **23,** 449-454.

Sheehy, A. M., Gaddis, N. C., Choi, J. D., Malim, M. H. (2002) Isolation of a human gene that inhibits HIV-1 infection and is suppressed by the viral Vif protein. *Nature* **418,** 646-650.

Sheehy, A. M., Gaddis, N. C., Malim, M. H. (2003) The antiretroviral enzyme APOBEC3G is degraded by the proteasome in response to HIV-1 Vif. *Nat. Med.* **9,** 1404-1407.

Sitas, F., Carrara, H., Beral, V., Newton, R., Reeves, G., Bull, D., Jentsch, U., Pacella-Norman, R., Bourboulia, D., Whitby, D., Boshoff, C., Weiss, R. (1999) Antibodies against human herpesvirus 8 in black South African patients with cancer. *N. Engl. J. Med.* **340,** 1863-1871.

Stremlau, M., Owens, C. M., Perron, M. J., Kiessling, M., Autissier, P., Sodroski, J. (2004) The cytoplasmic body component TRIM5alpha restricts HIV-1 infection in Old World monkeys. *Nature* **427,** 848-853.

Svoboda, J. (1961) The tumorigenic action of Rous sarcoma in rats and the permanent production of Rous virus by the induced rat sarcoma XC. *Folia Biol. (Praha)* **7,** 170-173.

Svoboda, J. (1962) Further findings on the induction of tumours by Rous sarcoma in rats and on the Rous virus-producing capacity of one of the induced tumours (XC) in chicks. *Folia Biol. (Praha)* **8,** 215-220.

Svoboda, J. (1964) Malignant interaction of Rous virus with mammalian cells in vivo and in vitro. *Natl. Cancer Inst. Monogr.* **17,** 277-298.

Svoboda, J. (2003) Postulation of and evidence for provirus existence in RSV-transformed cells and for an oncogenic activity associated with only part of the RSV genome. *Gene* **317,** 209-213.

Svoboda, J., Dourmashkin, R. (1969) Rescue of Rous sarcoma virus from virogenic mammalian cells associated with chicken cells and treated with Sendai virus. *J. Gen. Virol.* **4,** 523-529.

Svoboda, J., Chýle, P., Šimkovič, D., Hilgert, I. (1963) Demonstration of the absence of infectious Rous virus in rat tumour XC, whose structurally intact cells produce Rous sarcoma when transferred to chicks. *Folia Biol. (Praha)* **9,** 77-81.

Svoboda, J., Machala, O., Hložánek, I. (1967) Influence of Sendai virus on RSV formation in mixed culture of virogenic mammalian cells and chicken fibroblasts. *Folia Biol. (Praha)* **13,** 155-157.

Teich, N. M., Weiss, R. A., Martin, G. R., Lowy, D. R. (1977) Virus infection of murine teratocarcinoma stem cell lines. *Cell* **12,** 973-982.

Temin, H. M. (1964) Nature of the provirus of Rous sarcoma. *Natl. Cancer Inst. Monogr.* **17,** 557-570.

Temin, H. M., Rubin, H. (1958) Characteristics of an assay for Rous sarcoma virus and Rous sarcoma cells in tissue culture. *Virology* **6,** 669-688.

Temin, H. M., Mizutani, S. (1970) RNA-dependent DNA polymerase in virions of Rous sarcoma virus. *Nature* **226,** 1211-1213.

Towers, G. J., Hatziioannou, T., Cowan, S., Goff, S. P., Luban, J., Bieniasz, P. D. (2003) Cyclophilin A modulates the sensitivity of HIV-1 to host restriction factors. *Nat. Med.* **9,** 1138-1143.

Varmus, H. E., Weiss, R. A., Friis, R. R., Levinson, W., Bishop, J. M. (1972) Detection of avian tumor virus-specific nucleotide sequences in avian cell DNAs. *Proc. Natl. Acad. Sci. USA* **69,** 20-24.

Veselý, P., Weiss, R. A. (1970) Interactions between cells with different degrees of malignancy. In: *Microcinematography as a Research Method in Cytology,* ed. Hroch, M., pp. 235-239, Charles University, Prague.

Veselý, P., Weiss, R. A. (1973) Cell locomotion and contact inhibition of normal and neoplastic rat cells. *Int. J. Cancer* **11,** 64-76.

Veselý, P., Mikš, A., Novák, J., Boyde, A. (2003) Calibration and quantification of fast intracellular motion (FIM) in living cells using correlation analysis. *Scanning* **25,** 230-239.

Watkins, J. F., Dulbecco, R. (1967) Production of SV40 virus in heterokaryons of transformed and susceptible cells. *Proc. Natl. Acad. Sci. USA* **58,** 1396-1403.

Weiss, R. A. (1967) Spontaneous virus production from 'non-virus producing' Rous sarcoma cells. *Virology* **32,** 719-722.

Weiss, R. A. (1969) The host range of Bryan strain Rous sarcoma virus synthesized in the absence of helper virus. *J. Gen. Virol.* **32,** 511-528.

Weiss, R. A., Payne, L. N. (1971) The heritable nature of the factor in chicken cells which acts as a helper virus for Rous sarcoma virus. *Virology* **45,** 508-515.

Weiss, R. A., Biggs, P. M. (1972) Leukosis and Marek's disease viruses of feral red jungle flow and domestic fowl in Malaya. *J. Natl. Cancer Inst.* **49,** 1713-1725.

Weiss, R. A., Friis, R. R., Katz, E., Vogt, P. K. (1971) Induction of avian tumor viruses in normal cells by physical and chemical carcinogens. *Virology* **46,** 920-938.

Weiss, R. A., Mason, W. S., Vogt, P. K. (1973a) Genetic recombinants and heterozygotes derived from endogenous and exogenous avian RNA tumor viruses. *Virology* **52,** 535-552.

Weiss, R. A., Vesely, P., Sindelarova, J. (1973b) Growth regulation and tumour formation of normal and neoplastic rat cells. *Int. J. Cancer* **11,** 77-89.

Weiss, R. A., Boettiger, D., Murphy, H. M. (1977) Pseudotypes of avian sarcoma viruses with the envelope properties of vesicular stomatitis virus. *Virology* **76,** 808-825.

Weiss, R. A., Clapham, P. R., Cheingsong Popov, R., Dalgleish, A. G., Carne, C. A., Weller, I. V., Tedder, R. S. (1985a) Neutralization of human T-lymphotropic virus type III by sera of AIDS and AIDS-risk patients. *Nature* **316,** 69-72.

Weiss, R. A., Teich, N. M., Varmus, H. E., Coffin, J. (1985b) *RNA Tumor Viruses*, eds. Weiss, R. A., Teich, N. M., Varmus, H. E., Coffin, J., pp. 1396, 1233, Cold Spring Harbor Laboratory Press, New York.

Závada, J. (1972) VSV pseudotype particles with the coat of avian myeloblastosis virus. *Nat. New Biol.* **240,** 122-124.

Závada, J., Dickson, C., Weiss, R. (1977) Pseudotypes of vesicular stomatitis virus with envelope antigens provided by murine mammary tumor virus. *Virology* **82,** 221-231.

Závada, J., Závadová, Z., Pastorek, J., Biesova, Z., Ježek, J., Velek, J. (2000) Human tumour-associated cell adhesion protein MN/CA IX: identification of M75 epitope and of the region mediating cell adhesion. *Br. J. Cancer* **82,** 1808-1813.

Závadová, Z., Závada, J., Weiss, R. A. (1977) Unilateral phenotypic mixing of envelope antigens between togaviruses and vesicular stomatitis virus or avian RNA tumour virus. *J. Gen. Virol.* **37,** 557-567.