Preisverleihung 2000

STIFTUNG PROFESSOR DR. MAX CLOËTTA

Heft Nr. 28

Prof Dr Giuseppe Pantaleo
«Mechanisms of human immunodeficiency virus (HIV) escape from the immune response»

Dr Brian A. Hemmings
«Protein kinase B (PKB/Akt) – a common element in multiple signaling pathways involved in insulin signaling, cell survival and cancer»

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VORWORT

Professor Dr. Alex F. Muller, Präsident

Ganz herzlich begrüsse ich Sie zur heutigen 27. Preisverleihung der Stiftung Prof. Dr. Max Cloëtta. Immer war es mir eine grosse Ehre und Freude, Sie zur jährlichen Feier willkommen zu heissen. Die heutige wird mir in spezieller Erinnerung bleiben, weil ich zum letzten Mal als Präsident der Stiftung zu Ihnen spreche. Die Preisverleihung ist immer ein besonderes Ereignis. Es freut uns, junge Kollegen für ihre hervorragenden wissenschaftlichen Leistungen auszeichnen zu dürfen. Der Stiftungsrat bemüht sich immer um eine ausgewogene, wissenschaftlich begründete Auswahl.

Die beiden Preisträger von heute abend haben, jeder auf seine Art, ein fundamentales Problem bearbeitet. Mit ihrer Forschung sind sie zu Resultaten gekommen, die schliesslich auf eine Erklärung, eine Lösung eines klinisch wichtigen Problems oder die Antwort auf eine bekannte Frage hinauslaufen. Die Stichworte sind auch den medizinischen Laien unter uns bestens bekannt: es geht um Diabetes einerseits und Aids anderseits.

Beiträge, wie sie unsere beiden Preisträger leisten, werden der Medizin weiterhelfen; sie verschaffen uns Mittel und Verfahren, die einerseits das Verständnis erweitern und anderseits in Therapie und Prävention neue Wege aufzeigen. Je grundlegender, je zielbewusster das ärztliche Denken und Vorgehen, desto rascher, präziser und treffender Diagnose und Therapie!

Wenn wir nun diesen Gedanken logisch weiter entwickeln, könnten wir zum Schluss kommen, dass diese Forschung den Aufwand und damit auch die Kosten für Diagnose und Therapie eigentlich zum Sinken bringen sollte. Mit dieser kühnen Behauptung wage ich mich aufs Glatteis, dessen bin ich mir bewusst. Denn leider ist, wie wir wissen,

das Gegenteil der Fall; die Kosten unseres Gesundheitswesens klettern in kaum mehr finanzierbare Höhen.

Ist dem wirklich so? Ist dies in allen Ländern der Fall? Oder gibt es Ausnahmen? Wenn ja, warum?

Einer Statistik der OECD (Organization for Economic Cooperation and Development) entnehmen wir folgende relevanten Daten:
Weltweit stiegen zwischen 1960 und 1990 die Kosten des Gesundheitswesens in allen Ländern. Ab 1990 erfolgte eine deutliche Trendwende. Gemessen am Bruttosozialprodukt hatten Deutschland und die Schweiz weltweit, sogar noch vor den Vereinigten Staaten von Amerika, die grösste Zunahme zu verzeichnen. In einzelnen Ländern wie z.B. Dänemark, Finnland, Norwegen, Schweden, Irland und Italien, sanken die Kosten sogar. Andernorts – auch in Amerika, nicht aber in Deutschland und der Schweiz – zeigt sich die Tendenz oder mindestens ein Bestreben zur Stabilisierung. Die Liste der Pro-Kopf-Ausgaben wird angeführt von den Vereinigten Staaten, dies infolge ihrer technisch hochstehenden und defensiven Medizin. Mit Aufwendungen von 4270 $ pro Kopf und Jahr wird der Durchschnittswert der 23 OECD-Staaten (2000 $) um mehr als das Doppelte übertroffen. An zweiter Stelle folgt auf dieser Liste bereits die Schweiz.

Im heutigen Zeitpunkt, im Spannungsfeld der Diskussionen über TarMed, Numerus Clausus, Praxis-Zulassungsbewilligungen, Grundversorgung versus Spezialisten-Medizin etc. sind die Erhebungen der OECD ernst zu nehmen. Sie müssen, wenn immer möglich «sine ira et studio», zu fruchtbaren, ausgewogenen Überlegungen und dann zu allgemein anerkannten, akzeptablen Lösungen führen.

Wir alle sind uns bewusst, dass eine der grossen Schwierigkeiten der praktischen Medizin darin besteht, aus der Fülle des medizinisch-technischen Angebotes die sachdienlichste, sicherste und dennoch kostengünstigste Auswahl zu treffen. Eine öfters überflüssige Quantität an Massnahmen lockt; dieser Versuchung zu widerstehen ist nicht einfach, die vielgepriesene Qualitätskontrolle ausserordentlich schwierig. Ich wage zu behaupten, dass im heutigen Zeitpunkt die ärztliche Versor-

gung an einem einmaligen Kreuzweg steht. Die Quadratur des Zirkels von Ärzten, Öffentlichkeit, Versicherungen und Staat darf auf keinen Fall als unlösbare Aufgabe betrachtet, sondern muss angegangen werden! Die offensichtlichen Schwierigkeiten dürfen auf keinen Fall als Alibi für Untätigkeit dienen. Die Stunde hat geschlagen, oder wie einer der grossen Zürcher Lehrer, Professor Wilhelm Löffler, zu sagen pflegte: «Der richtige Moment in der Medizin ist nur ein Augenblick!»

Nach diesen durch die Aktualität der Problematik bedingten Überlegungen wenden wir uns nun wieder unseren Preisträgern zu. Wir freuen uns, Ihnen heute abend einmal mehr zwei hervorragende Forscher vorzustellen. Der Stiftungsrat der Stiftung Prof. Dr. Max Cloëtta ist überzeugt, mit ihrer Auszeichnung einen wichtigen Beitrag zur Schweizerischen Wissenschaftspolitik zu leisten.

Als ersten darf ich Ihnen Herrn Professor Giuseppe Pantaleo vorstellen. Sein Spezialgebiet ist die Immunologie, vor allem im Zusammenhang mit Aids. Mit seinen 44 Jahren kann er eine eindrückliche Publikationsliste vorlegen. Unser Preisträger ist zurzeit Leiter der Division für Immunologie und Allergologie am Centre Hospitalier Universitaire Vaudois (CHUV) in Lausanne. Es freut mich, Herrn Professor Michel Glauser, Dekan der Medizinischen Fakultät der Universität Lausanne, bei uns begrüssen zu dürfen. Ich danke ihm herzlich, dass er sich bereit erklärt hat, die Laudatio für seinen Lausanner Kollegen zu halten. Schon früher hat er sich für den Preisträger engagiert: er war massgeblich daran beteiligt, dass Professor Pantaleo aus den Vereinigten Staaten in die Schweiz zurückkehrte, um in unserem Land seine Forschungsarbeiten voranzutreiben.

Dr. Brian A. Hemmings ist Senior Scientist am Friedrich Miescher-Institut in Basel. Es waren unter anderem seine hervorragenden Leistungen auf dem Gebiete des Insulin Signalling, die den Stiftungsrat bewogen, ihn als Cloëtta-Preisträger auszuzeichnen. Es freut mich, Herrn Professor Emmanuel Van Obberghen aus Nizza begrüssen zu dürfen und ihm unseren aufrichtigen Dank dafür auszusprechen, dass er die lange Reise auf sich genommen hat, um die Laudatio für Herrn Dr. Hemmings zu halten.

Neben der Verleihung des Cloëtta-Preises vergeben wir dieses Jahr wiederum Forschungsstipendien für den künftigen akademischen Nachwuchs in der Schweiz. Eine äusserst wertvolle und stimulierende Tätigkeit für einen Stiftungsrat!

Abschliessend gestatten Sie mir ein Wort des Dankes an die Mitglieder des Stiftungsrates und dessen Medizinischen Ausschusses. Es war eine schöne und anregende Aufgabe, die ich gemeinsam mit Ihnen anpacken durfte. Ich bin Ihnen sehr dankbar für Ihre wertvolle, hilfreiche und immer zuverlässige Unterstützung. Unsere Zusammenarbeit wurde dadurch entscheidend bereichert und verbessert. Ihnen und der Stiftung Prof. Dr. Max Cloëtta wünsche ich viel Freude und Erfolg für die kommenden Jahre.

Was eine Stiftung entscheidend beeinflusst, ist ihre Infrastruktur, d.h. ihr Sekretariat. Die Stiftung Prof. Dr. Max Cloëtta ist in dieser Hinsicht äusserst privilegiert. In Frau Brigitt Schai und Frau Irène Thomann haben wir zwei ganz hervorragende, aussergewöhnliche Mitarbeiterinnen. Alle unsere Jahre der Zusammenarbeit waren gekennzeichnet durch Qualität, Wirksamkeit und Leistungsfähigkeit, kombiniert mit Freude und Humor! Für all dies möchte ich Ihnen meinen aufrichtigen Dank aussprechen.

C'était une belle et enrichissante aventure!

DANKESWORT AN DEN ABTRETENDEN PRÄSIDENTEN DER STIFTUNG, HERRN PROF. DR. ALEX F. MULLER

Prof. Dr. Max M. Burger

Er kam, sah sich um und begeisterte.

Jedenfalls drang die Kunde von deiner Rückkehr vom Massachusetts General Hospital anfangs der fünfziger Jahre in mein Doktorandenlabor in Zürich. Mein an und für sich sehr zurückhaltender Mentor am Pharmakologischen Institut, Heinrich Langemann, beschrieb dich als einen der wenigen Kliniker, die im Mekka der damaligen klinischen Forschung in Boston den Spagat zwischen Medizin und Biochemie mit Erfolg gemacht hatten. Aus der Froschperspektive eines mit biochemischen Methoden bastelnden Doktoranden war das die Bestätigung, dass die künftige klinische Forschung ohne ein Verständnis der molekularen Prozesse nicht mehr auskommen wird. Du wurdest, kaum zurückgekehrt, auch mit deiner Klinik in Genf zum Beispiel einer Erneuerung, die kurz danach Zürich und andere Kliniken in der Schweiz mit erfasste. Andere «Harvard-Schweizer» wie Albert Renold kamen dann auch zurück nach Genf oder wie Ruedi Froesch zu Alex Labhart in Zürich.

Deine Mineralstoffwechsel-Forschungsgruppe in Genf gehörte bald zur Spitze in Europa, weshalb der Forschungsrat in Bern deine Dienste nicht entbehren konnte. Dort lernte ich Mitte der siebziger Jahre deine wissenschaftlich kompromisslose, sonst aber vermittelnde und beispielhaft kollegiale Art und Weise kennen und war retrospektiv erstaunt, dass Alfred Pletscher dich «Tongue in Cheek» mir als Zar Alexander von Genf vorgestellt hatte. Der Kreml hat ja auch dicke Mauern, dachte ich, und die Romanoffs hatten vielleicht auch Jekells und Hydes drinnen und draussen, war dann aber beruhigt, als Albert Renold bei einem Glas Bier dir Krone und Titel aberkannte als eine unberechtigte Mär einiger deiner Oberärzte. Shame on them! Oder wo Rauch war, motteten doch noch Glutrestchen?

Zum ersten Mal

Dank des Verzichtes von Herrn Dr. Hans-Ulrich Doerig, der als hervorragender Bankfachmann traditionsgemäss den Vorsitz des Stiftungsrates von Herrn Dr. Alfred Hartmann hätte übernehmen können, wurdest du zum ersten Mal in der Geschichte unserer Stiftung in deiner Funktion als Vorsitzender des medizinischen Ausschusses nun gleichzeitig auch verantwortlich für die gesamte Stiftung. Du hast diese Aufgabe vor fünf Jahren mit deinem charakteristischen Elan in Angriff genommen. Mit deinen Erfahrungen vom Nationalfonds und mehreren anderen Stiftungen wie der Marcel Benoist-Verwaltungskommission, die du viele Jahre geleitet hast, waren dir viele Probleme der finanztechnischen Forschungsverwaltung in der Praxis bekannt. Nicht zuletzt wegen dieser Doppelfunktion als Vorsitzender des medizinischen Ausschusses und als Präsident der Stiftung sind dir zwei wichtige Neuerungen zu verdanken. So gibt es zwischen den Schweizer Universitäten zum Teil eklatante Ungleichheiten, manchmal sogar Ungereimtheiten in bezug auf Forschungsstellensaläre. Insbesondere bestehen zum Teil auch mehr als störende Unterschiede zwischen den durch die verschiedenen Universitätsverwaltungen errechneten und belasteten Sozialabgaben. In deiner Amtszeit hast du diese, viele Schweizer Stiftungen beschäftigende Problematik beispielhaft gelöst.

In den vergangenen Jahren haben die Anfragen nach sogenannten Überbrückungskrediten massiv zugenommen. Wir verstehen die Notwendigkeit, kurzfristig Geldmittel beschaffen zu müssen, wenn eine Stelle ausläuft und eine neue Stelle nicht nur mittelfristig in Aussicht steht, sondern zugesagt wurde. Eine Brücke braucht nun einmal mindestens zwei Pfeiler, und so mussten klare, allgemeingültige Richtlinien für Überbrückungskredite formuliert und festgelegt werden. Die von dir erarbeiteten Grundlagen wurden 1997 verabschiedet und haben sich inzwischen als nicht nur praktikabel, sondern auch als Sitzungszeit sparende und vor allem die Verwaltung erleichternde Massnahmen erwiesen.

M.D.- Ph.D.-Programm und Erhaltung eines relevanten Anteils an klinischer und medizinisch orientierter Forschung

Die Erhaltung unserer Beiträge an M.D.-Ph.D-Studenten lag dir sehr am Herzen. Dieses überaus wirkungsvolle nationale Programm, das du mit begründet hast, sichert den Nachwuchs an forschungsorientierten jungen Medizinern. Der Zufluss in diesen Pool war schon immer prekär und hat durch dieses M.D.-Ph.D.-Programm nun eine partielle Stabilisierung erfahren. Aus diesem Programm rekrutiert sich doch immerhin ein Teil der späteren Spitzenforscher an den klinischen Zentren unseres Landes. Wir werden uns bemühen, diese Nische der Nachwuchsförderung weiterhin zu füllen. Insbesondere werden wir aber dein Hauptanliegen vor Augen behalten, die klinische und die medizinisch orientierte Forschung nicht durch die rasch wachsende molekularbiologisch orientierte Forschung einzuschränken, sondern bei der Preiszusprache und den Forschungsstellengesuchen die beiden Gebiete voneinander zu trennen.

Die gesamte Stiftung wünscht dir einen doch langsam weniger unruhigen Ruhestand als bis anhin und dankt dir für deinen altersungemässen, höchst vitalen Beitrag über die vergangenen Jahre.

LAUDATIO FOR PROFESSOR GIUSEPPE PANTALEO

Prof Dr Michel P. Glauser

When I first met with Giuseppe Pantaleo in Washington DC, did I realize or ever imagine that a few years later he would be chief of the Division of Immunology and Allergy at our University Medical Center, that he would lead such a wonderful team of scientists around him, be a key player within the AIDS community in Europe and around the world, and more important, a pioneer and a leading authority in the understanding of the immunology of HIV infection? I remember vividly that evening on a terrace in a small Italian bistro in Bethesda, famous because the Clintons used to drop by for enjoying the wine list and endless varieties of Grappa. This was at the time when the NIH was offering Dr Pantaleo a permanent position in Toni Fauci's lab, when he was also offered a position at the Gladstone Institute in San Francisco to develop an new avenue of research in the immunology of HIV infection. The chance for a moneyless Lausannois to attract the rising star to the Leman lake-side looked totally irrealistic at first glance. The chance for us however was hidden in the Pantaleo's dream to get back to Europe and particularly to Lausanne so as to benefit from the immunological excellence existing there.

Giuseppe Pantaleo was born in Bari, Italy in 1956. He went to school there, followed by the University of Bari Medical School, where he received the degree in medicine in 1980, with full marks and honors (110/110 cum laude). His thesis for the MD degree was entitled «Defects in chemotactic function of monocytes and granulocytes in patients with breast cancer»: already an interest in host defenses, and in patients with immune defects such as those existing in cancer patients! This was followed by an internship and residency in internal medicine, with a particular accent on hematology and oncology. During this training, G. Pantaleo was in a Department whose many interests were in patients with autoimmune diseases, multiple mycloma, a Department

under the leadership of Prof Franco Dammacco, who introduced him to the field of immunology. After obtaining a board in clinical hematology (with full marks and honors, again, 50/50 cum laude) at the University of Bari in 1993, he was awarded an EMBO fellowship to visit the Ludwig Cancer Research Institute in Lausanne, and was subsequently offered a position in the Group of Human Immunology, headed by Lorenzo Moretta. During the first 2 – 3 years of his stay there, Dr Pantaleo learned the basics of immunology of T-lymphocytes, their characterization, and was mainly involved in the development of a limiting dilution cloning system of human T-lymphocytes (at that time, this was the first such system developed in humans). The development of this system was critical for the subsequent studies aimed at the phenotypic and functional characterization of circulating human T-lymphocytes, in particular of the CD4 and CD8 subpopulations which has become so important.

These functional studies permitted to determine the involvement of different T-cell surface molecules in T-cell-mediated functions, which naturally led Dr Pantaleo to get into the study of the regulatory mechanisms existing among the different pathways involved in T-cell activation. From 1985 to 1987, Dr Pantaleo was amongst the pioneers in the delineation of the molecular and biochemical mechanisms involved in signal transduction in human T-lymphocytes. He was the first to characterize the signal transduction pathways of CD2 and CD28 molecules and to identify different patterns of signaling in different T-cell subsets.

At the end of 1987, he was back to Bari, as Assistant Professor in the Department of Internal Medicine, with the task of developing research in clinical immunology in patients with various immune disorders and lymphomas. The goal was to use the tools he had learned in Lausanne to better understand the dysregulations in these patients. In early 1988, he was invited for 3 months as a consultant in the Laboratory of Dr Anthony S. Fauci at the NIH for a focused project of T-lymphocyte cloning, in patients with HIV infection. This is when he was offered a position at this prestigious place.

In 1989, he entered the Laboratory of Immunoregulation of the National Institute of Allergy and Infectious Diseases as Visiting Associate. Building on the observations he made during his brief stay the year before, he was the first to demonstrate, in 1989, the defective clonogenic potential of CD8+ T-lymphocytes, and the selective defect of the function of HIV-specific cytotoxic T-lymphocytes. Strangely enough, these functional deficiencies of the CD8+ specific T-lymphocytes were associated with an augmented activation of this type of cells. Clearly, for an immunologist by training like Dr Pantaleo, such an observation naturally led him to try to understand the early immunological events during HIV infection, in particular in those organs where antigens are presented to the immune system, in other words in the lymphatic tissue.

We should remember here that in the late 80's, early 90's, the dogma of the natural history of HIV infection was that there was first a large burst of viral production during the primary infection followed by a long period of silent, immunologically controlled infection (the so called «latency»), and only when the immune system was «exhausted» did infection and viral replication resume, with a fall in CD4 lymphocytes and the development of a profound state of immunodeficency. To his great surprise, as soon as he drew his attention to the lymphatic organs, Dr Pantaleo discovered that even during the so called clinical latency, there was full, continued viral replication. By combining the methodology of the polymerase chain reaction for the quantitation of viral burden (i.e., HIV DNA copies) and viral replication with that of in situ hybridization for virus distribution, he discovered that lymphoid organs function as a major reservoir for HIV, and that active virus replication occurs throughout the course of HIV disease, in other words that the clinical latency was a misconception. These findings have been of critical importance for developing a coherent model of progression of HIV disease, and have completely changed the clinical and therapeutic management of HIV infection. His Nature paper in 1993 was the 2nd most cited paper in research in 1993.

In 1994, in a second paper in Nature, he demonstrated that during the mononucleosis like primary infection, up to 40% of the circulating

CD8 T-lymphocytes are specific for HIV, but have a severe maturation block, and only 1% have a fully efficacious cytotoxic function. This is in contrast of course to other viral infections like EBV and CMV, where there is also such a brisk and major CD8 expansion during primary infection, but with fully functional cells that permit the organism to subdue the viral proliferation, and get into a true latency phase.

After these important discoveries, and the development of this superb and productive career, there was obviously a great interest for many places to attract Dr Pantaleo, as I alluded to earlier. However, the chances for the «Suisse romande» to attract Dr Pantaleo appeared rather small, but two concurrent «synergistic» factors made the dream come through. The first was the existence of a foundation in Lausanne and Geneva, the Foundation Giorgi Cavaglieri, whose mission is to promote medical research in the field of immunology and oncology. Its board was very receptive to the suggestion to sponsor the development of a center of excellence in AIDS research, that would be based both in Lausanne and Geneva. To foster this «Centre lémanique de recherche sur le SIDA», the Foundation Giorgi Cavaglieri attributed a five year grant to the Faculties of Medicine to support the salaries of Dr Pantaleo in Lausanne and of Dr Didier Trono in Geneva. Dr Trono is a former Geneva MD trainee, who had a superb career in basic virology, mainly in HIV research at the Whitehead Institute in Boston and at the Salk Institute in La Jolla.

To match this generous grant, Dr Charles Kleiber, at the time Director of the Hospital in Lausanne and now Secretary of State for Education and Research in Berne, entirely financed the building of a superb lab with a P3 facility in what was the pediatric surgery operating suite.

Dr Pantaleo moved to Lausanne in September 1996. Rapidly, he was able to develop a very efficient group of researchers that concentrated of several avenues of research. First, Dr Pantaleo has continued to provide important contributions to the understanding of the immunopathogenesis of HIV infection. Contrary to the widely held belief that the vanishing of CD4+ T-lymphocytes was due to their over production and turnover, he showed that CD4 loss during AIDS progression was in

fact due to a blocked production rather than an exaggerated destruction. Second, he continued a program that he started at NIH, aimed at evaluating the effect of antiviral therapy in lymphoid tissues. His group has been involved in the understanding of the restoration of the immune functions that accompany the recovery of CD4 T-lymphocytes following highly active antiretroviral therapy.

Third, he has started a program aimed at developing novel concepts of immune-based therapeutic interventions in HIV infection. In one of these approaches, the goal is to reduce the proportion of cycling CD4 cells, i.e. those activated cells that support active virus replication. This has led to a pilot study using mycophenolic acid jointly together with an antiviral therapy, the hypothesis being that the intervention would prevent both the CD4 activation and the viral multiplication, without affecting the CD8 cytotoxic response. Results from this study show that this approach is promising.

In conclusion, it has been a personal pleasure and honor for me to present to you, Professor Giuseppe Pantaleo, who was awarded the Cloëtta award today. He will contribute, I am certain, to the prestige of this award both within Switzerland and abroad. Dr Pantaleo is a typical modern scientist, and an example for the generations to come. He is a skilled experimenter in the lab, constantly developing the most sophisticated technical platforms necessary to explore the fine understanding of immune functions, and to stay at the fore front of research. He is a talented and rigorous clinical investigator, quickly introducing to the patient the boldest hypothesis that are suggested by the experimental observations. Finally, and equally importantly, he is a true entrepreneur, constantly moving around the world to find new opportunities so as to gather the funding necessary for expanding his research.

I am sure you have made a good choice, and wish Giuseppe Pantaleo a continued success.

LE PRIX LOUIS-JEANTET 2001
EST DÉCERNÉ À

MONSIEUR

GIUSEPPE PANTALEO

PROFESSEUR EN MÉDECINE

NÉ EN 1955 À BARI (ITALIE)
PROFESSEUR À L'UNIVERSITÉ DE LAUSANNE
ET CHEF DE LA DIVISION D'IMMUNOLOGIE ET D'ALLERGIE AU
CENTRE HOSPITALIER UNIVERSITAIRE VAUDOIS, LAUSANNE
POUR SES REMARQUABLES TRAVAUX DANS LE DOMAINE DE
L'IMMUNOLOGIE ET DE L'IMMUNOPATHOLOGIE DU SIDA.

ZURICH, LE 15 NOVEMBRE 2001

AU NOM DU CONSEIL DE FONDATION:

LE PRÉSIDENT: LE VICE-PRÉSIDENT:

UN MEMBRE:

Prof. Dr. Giuseppe Pantaleo

CURRICULUM VITAE

Name:	Giuseppe Pantaleo, M.D.
Date and Place of Birth:	June 17, 1956, Bari, Italy
Citizenship:	Italy

Education:

1983	Specialized in Clinical Hematology University of Bari Bari, Italy
1980	M.D. (Surgery), University of Bari, Italy, Thesis: «Defects in chemotactic function of monocytes and granulocytes in patients with breast cancer». (110/110 et laude)
1978 – 1979	Student Internship, Department of Internal Medicine, University of Bari, Italy
1974	B.A., Bari, Italy

Employment:

1999 (Aug.) – to date	Professor of Medicine and Chief Division of Immunology and Allergy, Department of Medicine, Division of Infectious Diseases, Centre Hospitalier Universitaire Vaudois (CHUV), Lausanne, Switzerland
1996 (July) 1999 (Aug.)	Professor of Medicine and Chief Laboratory of AIDS Immunopathogenesis, Department of Medicine, Division of Infectious Diseases, Centre Hospitalier Universitaire Vaudois (CHUV), Lausanne, Switzerland
1991 – 1996 (July)	Senior Investigator, Laboratory of Immunoregulation

	National Institute of Allergy and Infectious Diseases National Institutes of Health Bethesda, Maryland
1989 – 1991	Visiting Associate Laboratory of Immunoregulation National Institute of Allergy and Infectious Diseases National Institutes of Health Bethesda, Maryland
1988 (Feb./March)	Scientific Consultant Laboratory of Immunoregulation National Institute of Allergy and Infectious Diseases National Institutes of Health Bethesda, Maryland
1987 (Nov.) – 1988	Assistant Professor Department of Internal Medicine University of Bari Bari, Italy
1985 – 1987	Postdoctoral Fellow Ludwig Institute for Cancer Researcher Lausanne, Switzerland
1984	Postdoctoral Fellow Department of Cellular Biology Suisse Institute for Cancer Research Lausanne, Switzerland
1983 – 1984	Postdoctoral Fellow Ludwig Institute for Cancer Research Lausanne Branch Lausanne, Switzerland
1982	Recipient of EMBO, Fellowship Ludwig Institute for Cancer Research Lausanne Branch Lausanne, Switzerland
1980 – 1981	Resident Department of Internal Medicine University of Bari Bari, Italy

Research activities:

Human T cell cloning
Human T cell phenotypic and functional analysis
Role of chemokine receptors in T cell traffic
T cell activation, differentiation and memory
Immunopathogenesis of HIV infection
HIV distribution in different anatomic comparments
Immune-based therapeutic strategies

Affiliations:

Swiss Society of Allergology and Immunology
Swiss Society of Infectious Diseases

Editorial activities:

Human Immunology: Board of Reviewing Editors
Journal of Human Virology: Board of Reviewing Editors

AIDS, Blood, European Journal of Immunology, Journal of AIDS, Journal of Clinical Investigation, Journal of Experimental Medicine, Journal of Immunology, Journal of Infectious Diseases, Journal of Virology, Nature Medicine, Proceeding National Academy of Science: Ad hoc reviewer

Honors:

1993	National Institutes of Health Award of Merit
1995	Public Health Service Special Achievement
1996	Academia dei Lincei – Award in Basic and Clinical Virology (Guido Lenghi and Flaviano Magrassi Foundation).
1996	National Institutes of Health NIH Director's Award
1999	Pfizer Research Prize in Clinical Research in Infectious Diseases

MECHANISMS OF HUMAN IMMUNODEFICIENCY VIRUS (HIV) ESCAPE FROM THE IMMUNE RESPONSE

Giuseppe Pantaleo

The acquired immunodeficiency syndrome (AIDS) was identified as a new disease in 1981 (1,2) and, its aetiological agent, the human immunodeficiency virus (HIV), was discovered in 1983 (3). Two types of HIV, i.e. HIV-1 and HIV-2, have been identified (4). Both HIV-1 and HIV-2 have similar structures, tropisms and disease outcome, although they have limited shared nucleotide sequence homology (42%) and limited antigenic cross reactivity (5). HIV is predominantly transmitted via sexual activity, the exchange of blood or body fluids contamined by the virus, and from mother to child. CD4+ T lymphocytes, monocytes and macrophages are the major targets of HIV.

AIDS is a worldwide epidemic. Estimates from the World Health Organization indicate that more than 30.000.000 persons are infected with HIV worldwide, and that >95% of HIV infections are confined in subdeveloped countries where the recently developed potent antiretroviral drug combinations are not available. Therefore, the development of preventative vaccines appears the only effective strategy for fighting the AIDS epidemic. The development of effective preventative AIDS vaccines requires, however, the delineation of the HIV-specific immune response and, in particular, the identification of those components of the immune response which are critical for the control of virus replication and spreading, and the development of immunization strategies that may generate protective virus-specific immune responses.

In the last 10 years my research activity has been centered on the investigation of the immunopathogenic mechanisms of HIV infection. In this review, I will first briefly discuss the clinical course of HIV infection and the biological characteristics of HIV. Then I will examine the general concepts of antiviral immunity and how these concepts apply

to the biological characteristics of HIV. Finally, I will discuss a series of immunologic and virologic mechanisms developed by HIV to escape the immune response and the abnormalities that render the HIVspecific immune response poorly effective in the control of HIV infection.

Clinical course of HIV infection

The clinical course of HIV infection generally includes three phases or stages: a) primary infection, b) clinical latency, and c) AIDS-defining illness (Figure 1) (6-8). Primary infection may be associated with a mononucleosis-like syndrome. Due to the lack of specificity and the variable severity of the clinical syndrome, and to the fact that the diagnostic blood test for HIV infection (detection of HIV antibody) may be negative early on, primary infection goes generally unnoticed. From a virologic standpoint, primary infection is characterized by a burst of viremia (up to 10^7 HIV RNA copies per ml of plasma) and high levels of virus expression and HIV DNA copies in CD4+ T lymphocytes.

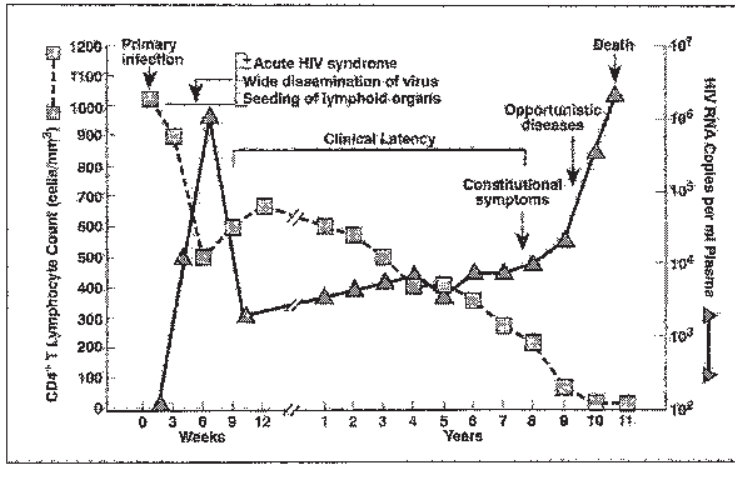

Figure 1. During the early period after primary infection there is widespread dissemination of virus and a sharp decrease in viremia followed by a prolonged period of clinical latency. The CD4 T-cell count continues to decrease during the following years, untill it reaches a critical level below which there is a substantial risk of opportunistic diseases.

Resolution of the clinical syndrome and downregulation of viremia generally occur 6-8 weeks following the appearance of symptoms and are associated with the emergence of HIV-specific immune responses. The downregulation of viremia marks the transition to the chronic clinically latent phase of HIV infection, which has a duration of 8-10 years. Although clinically silent, this phase is associated with high levels of virus replication in lymphoid tissues and with a progressive depletion of CD4+ T lymphocytes. The progression to AIDS-defining illness is characterized by CD4+ T cell counts below 200 cells per µl. This phase is complicated by opportunistic infections that are generally the cause of death.

Main forms of HIV in the body

In order to understand the characteristics of the immune response necessary to exert effective control of virus replication and spreading, it is important to delineate the biological forms under which HIV may persist in the body. There are three major forms of HIV in the body: a) cell associated virus; b) free virus; and c) follicular dendritic cells (FDC) associated virus (Figure 2) (9-11). With regard to the cell asso-

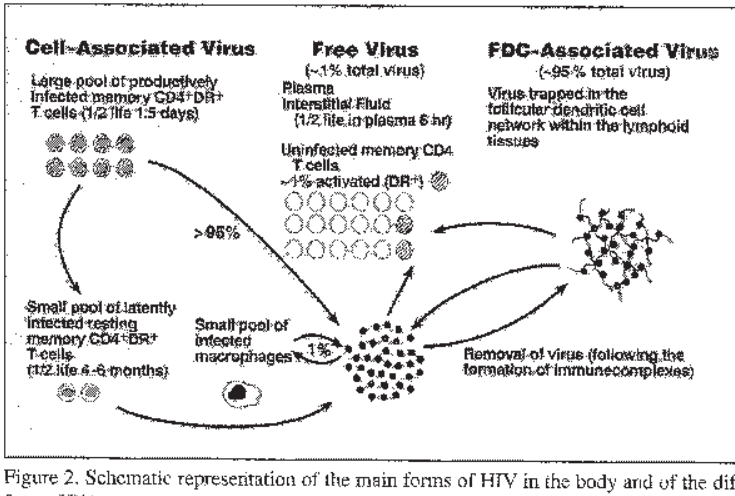

Figure 2. Schematic representation of the main forms of HIV in the body and of the different HIV compartments responsible for active virus production.

ciated virus, viral replication in activated memory CD4+ T lymphocytes supports most (98-99%) of the total virus production whereas monocyte/macrophage cells would be responsible for 1-2% of the total virus produced. A minor proportion of cell associated virus is represented by the small pool of latently infected resting CD4+ T cells containing replicating competent virus. Free virus (HIV in plasma and interstitial fluids) represents only 1% of the total virus. FDC associated virus represents most (>95%) of the total virus present in the body. FDC associated virus corresponds to HIV coupled with immunoglobulins and complement and trapped in the FDC network within the lymphoid tissue.

Effector mechanisms of antiviral immunity

The effector mechanisms of antiviral immuniy include both virus-specific humoral and cell-mediated immune responses (12). With regard to the humoral immune response, the protective component of this response is represented by the so called neutralising antibodies, i.e. antibodies which bind to free virus particles and prevent the transmission of the virus to the target cells (Figure 3). The protective component of the cell-mediated immune response is predominantly represented by cytotoxic T lymphocytes (CTLs), characterized by the expression of the surface molecule CD8 (Figure 3). CTLs are able to lyse virus infected target cells through the recognition of the complex formed by the virus-specific peptide plus the products of the major histocompatibility (MHC) class I antigen on the cell surface of infected cells. The efficacy of the antiviral effector mechanisms is strictly dependent upon the biological characteristics of the virus. In this regard, as proposed by Zinkernagel, a major role is played by the degree of cytopathicity, i.e. the ability to cause massive lysis of the target cells (12). The virus-specific humoral immune response, i.e. neutralising antibodies, is highly effective against cytopathic viruses which, by mediating lysis of the target cells, causes massive release of virus particles that can be neutralised by the specific antibodies whereas are poorly effective against non-cytopathic viruses (Figure 4). In contrast, due to the ability to lyse virus infected target cells, virus-specific cell-mediated immunity, i.e. CTLs, is highly effective exclusively against non-cytopathic viruses (Figure 4).

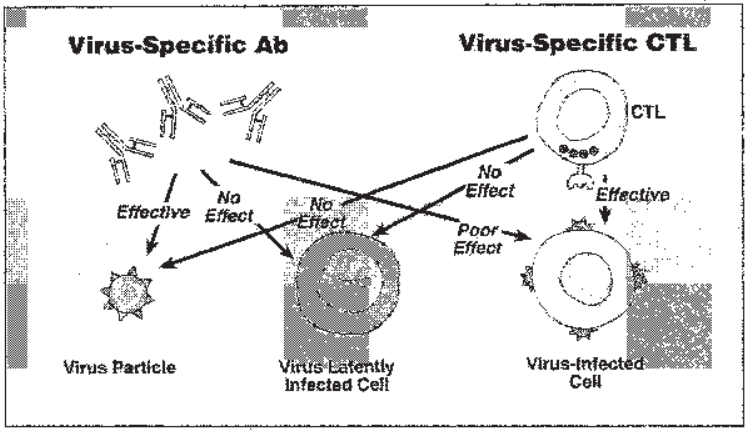

Figure 3. Protective components of the virus-specific humoral and cell-mediated immune responses. Effectiveness of the different types of antiviral immune response against the different forms of virus. Virus-specific Ab are effective against free virus particles and poor effective agains virus-infected cells (i.e. cell-associated virus). Virus-specific CTL are effective against virus-infected cells but have no effect against free virus particles. Both types of immune response are not effective against virus latently infected cells.

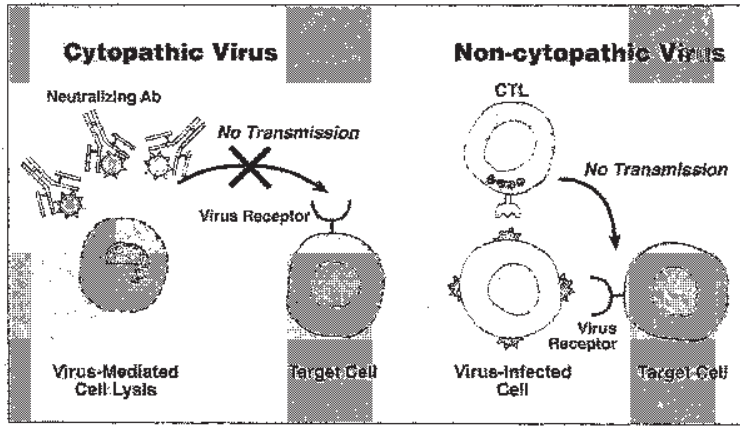

Figure 4. Schematic representation of the efficacy of the different types of the antiviral immune responses with regard to the biological characteristics of the virus, i.e. degree of cytopathicity. Neutralising Ab are effective against cytopathic viruses whereas CTL against non-cytopathic viruses.

Although HIV is highly cytopathic *in vitro*, there is little evidence that HIV is cytopathic *in vivo*. Therefore, as proposed by Zinkernagel, HIV should be considered as a non-cytopathic virus (13,14). Based on this hypothesis, HIV-specific cell-mediated rather than humoral immunity should play a critical role in the control of HIV replication and spreading. In the last 5 years, several studies have clearly demonstrated the importance of HIV-specific CD8+ CTLs as the primary effector mechanism for the control of HIV (15-17).

Mechanisms of HIV escape from the immune response

Recent studies have shown that viruses have evolved multiple strategies to evade the immune response and to establish a state of chronic infection (18-25). These include viral latency; inhibition of antigen processing and/or presentation; mutations in viral epitopes that compromise recognition by immunoglobulins or CTLs; viral mutations that alter binding to MHC or TCR molecules can lead to T cell anergy or peptide antagonism; and rapid clonal exaustion/deletion of the initially expanded virus-specific CD8+ CTL clones has been demonstrated in virus infections in mice.

The success of HIV in establishing chronic disease and in promoting a slow but progressive deterioration of the different components of the immune response is somewhat difficult to explain. Potent virus-specific cell-mediated and humoral immune responses can already be detected during the early days of primary HIV infection, and can persist for years without either preventing establishment of chronic infection or blocking HIV disease progression (6,15-17). Similar types of immune responses have been shown to be effective against other viral pathogens such as Epstein-Barr virus (EBV), influenza virus, Herpes viruses, and cytomegalovirus (CMV). Although EBV, CMV and Herpes viruses persist in the host, the antiviral immune response elicited is able to exert effective long-term control. What distinguishes HIV from EBV, CMV and Herpes viruses is that it has evolved several strategies at rapidly establishing continuous viral replication by interfering with protective immune responses. The sucess of these strategies depends

on four things: 1) the timing of pathogenic mechanisms; 2) the broad spectrum of effector components of the immune response that is targeted; 3) the ability of HIV to re-shape antiviral effector mechanisms into self-defense mechanisms; and 4) the impaired maturation of HIV-specific memory CTLs.

1. *Timing of pathogenic mechanisms*

Four important mechanisms involved in the early establishment of persistent infection and in viral escape from the immune response are active during primary HIV infection.

On the basis of the different usage of the CC chemokine co-receptor for entry into target cells, non-syncytium-inducing macrophage-tropic and syncytium-inducing T cell-tropic variants of HIV have been recently termed R5 and X4 strains (25), respectively. Importantly, R5 and X4 viral envelope can mediate biologically relevant transmembrane signals to CD4+ T cells (26,27). In the case of R5 envelope, this interaction results in activation and chemotaxis of these cells, at least *in vitro*. Therefore, the active recruitment of activated CD4+ T cells to the site of initial virus localization certainly leads to rapid spreading and amplification of HIV infection (Figure 5).

It is likely that simultaneously to the rapid spreading of HIV during primary infection occurs the formation of a pool of latently infected CD4+ T cells containing replication-competent HIV proviral DNA (28) (Figure 5). This event enables HIV to establish viral latency before the appearance of an HIV-specific immune response in the host, invariably leading to the establishment of chronic infection. Furthermore, these latently infected CD4+ T cells constitute a stable viral reservoir in which HIV remains sheltered from the effects of the host immune responses and anti-retroviral therapy.

HIV-specific proliferative responses are rapidly lost during primary HIV infection (Figure 5) (29). However, despite the loss of HIV-specific CD4+ proliferative responses, recent studies have indicated that

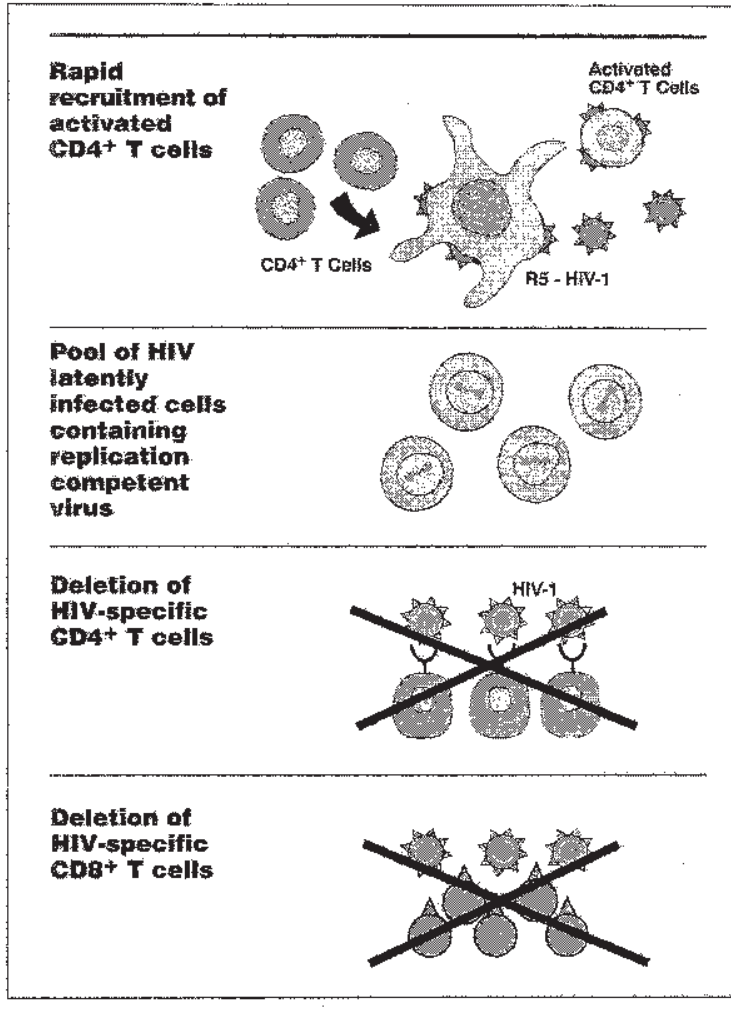

Figure 5. Timing of pathogenic mechanisms involved in the establishment of persistent infection. M-tropic (R5) HIV envelope expressed by infected Langerhans cells can mediate an activation/chemotaxis signal to CD4+ T cells leading to the rapid recruitment of these cells to the site of initial viral replication. A long-lasting reservoir of CD4+ T cells containing latent replication-competent provirus is rapidly established. Both CD4+ and CD8+ HIV-specific T lymphocytes may be rapidly depleted during primary infection.

HIV-specific CD4+ T cells might be significantly reduced in their frequency (30) or, more rarely, deleted at the time of primary infection, and that the functional defect of HIV-specific CD4+ T cells is both quantitative and qualitative.

Certain HIV-specific CTL clones that undergo massive clonal expansion might be deleted during primary HIV infection by a mechanism analogous to the clonal exhaustion that is observed in mice during acute lymphocytic choriomeningitis virus (LCMV) infection (24,31) (Figure 5). The early impairement of the virus-specific CTL response might have a major impact on both the short- and the long-term control of virus replication and disease progression. In fact, downregulation of viremia associated with primary infection has been temporally associated with the emergence of CD8+ HIV-specific CTLs, and correlations have been found between high levels of CTL activity and a slower rate of disease progression and lower levels of viral load (6,15-17).

2. *Spectrum of different components of the immune response targeted by HIV*

HIV might interfere with other cell types whose function is crucial for the generation of effective immune responses, i.e. monocytes/macrophages and dendritic cells. Interference with antigen presenting cells (APC) function might be the result of either quantitative depletion or suboptimal formation of MHC-antigenic peptide complexes. In this regard, it has been shown that the Nef protein of HIV can induce downregulation of cell-surface MHC class I expression which, in turn, might affect both generation of antigen-specific immune responses and the recognition of virus-infected target cells by CTLs (32,33) (Figure 6).

HIV-specific humoral immune responses can be detected readily during primary infection, but are mostly comprised of low-avidity Env-specific IgG antibodies that possess little or no neutralising activity (34,35). In fact, emergence of significant neutralising titers does not generally take place before transition to chronic HIV infection.

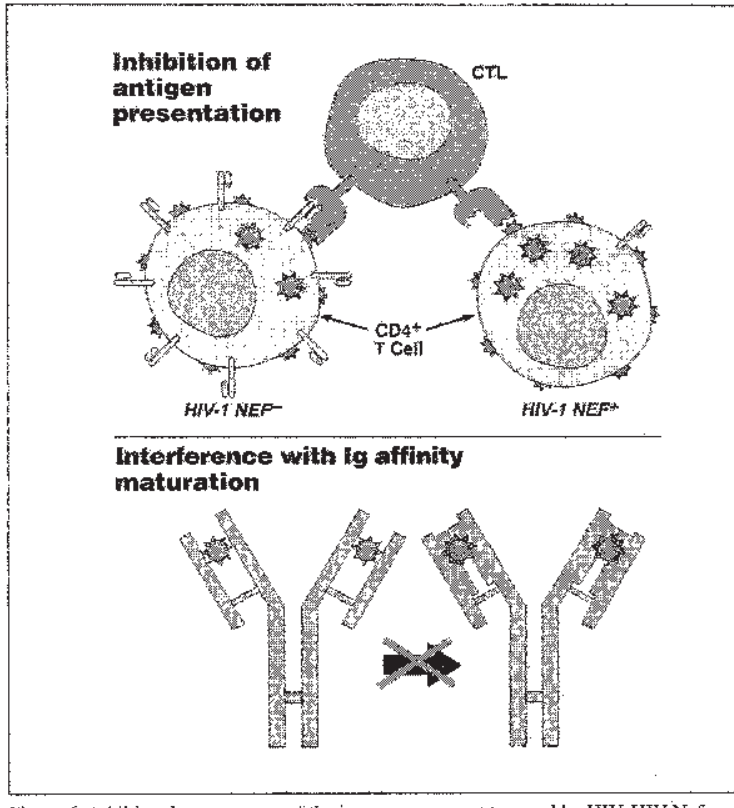

Figure 6. Additional components of the immune response targeted by HIV. HIV Nef gene product interferes with antigen presentation and cell killing by inducing downregulation of MHC class I expression at the surface of HIV infected cells. The delay in the generation and development of the antigen-specific neutralising antibody response may result from a direct and/or indirect interference with immunoglobulin (Ig) avidity maturation.

Infection with most Lentivirinae, including HIV, is characterized by a slow maturation of the antibody response (36). The reasons for this delay are poorly understood, but the early destruction/dysfunction of CD4+ T cells might result in the alteration of several parameters of the antibody response, such as circulating titer, avidity maturation, and neutralising activity (36,37) (Figure 6).

3. *Reshaping of antiviral mechanisms*

The frequent occurrence of CTLs escape mutants during chronic and primary HIV infection has been documented extensively (38-41), and non-synonymous nucleotide substitutions are selected preferentially in amino acid residues located within cognate CTLs epitopes (41). This indicates that the rapid emergence of CTLs escape mutants is actively driven by the selective pressure exerted by the cytotoxic virus-specific immune response. Therefore, although the emergence of CTLs responses is essential for the downregulation of viremia and for preventing disease progression, CTLs pressure itself appears to induce the selection of virus mutants capable of escaping the immune response (Figure 7).

Rapid spreading of HIV infection and high levels of virus replication occur in lymphoid organs (6). During primary infection, higher frequencies of *in vivo*-activated HIV-specific CTLs have been found in peripheral blood (42). These observations suggest that HIV-specific CTLs actively egress from lymph nodes into the circulation, thereby accumulating in a compartment where only low levels of HIV replication take place (Figure 7). Egress of activated antigen-specific CTLs from lymphoid tissue into the circulation is likely to be a physiological step that occurs after the generation of the immune response, to achieve a wide distribution of antigen-specific effector cells in different anatomical sites. However, in HIV infection it serves also as a mechanism for directing CTLs away from the primary site of viral replication.

Concentration of HIV virions on the surface of FDC in the germinal centers of lymphoid tissue occurs during the transition from the acute to the chronic phase of HIV infection (Figure 7) (43,44). Formation of immune complexes (i.e. HIV bound with immunoglobulin and complement) and their seeding in the FDC network are physiological mechanisms generally devoted to the clearance of the pathogen within the reticuloendothelial system, and to the generation of effective immune responses. However, in HIV infection these mechanisms lead to the formation of a stable reservoir of infectiuous virions that form a continuous source for the infection of CD4+ T cells, and to a chronic inflammatory reaction that ultimately results in the destruction of lymphoid tissue.

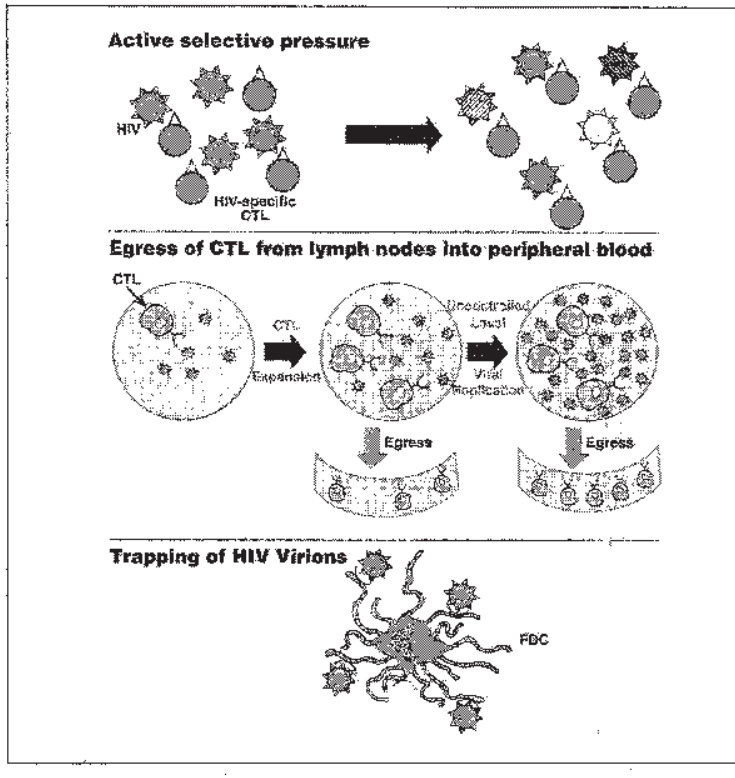

Figure 7. Reshaping of antiviral mechanisms. The intense selective pressure exerted by HIV-specific CTLs causes may cause rapid emergence of CTLs escape variant during primary infection. The active egress of HIV-specific CTLs from lymph nodes into the peripheral circulation may impair the clearance of HIV-infected cells in lymphoid tissue and allow HIV to establish persistent infection. Trapping of HIV within the FDC network leads to the concentration of infectious virions and to a chronic inflammatory reaction that might contribute to the destruction of lymphoid tissue.

4. *Impaired maturation of HIV-specific memory CTLs*

The expression of the chemokine receptor CCR7 defines, at least *in vitro*, distinct subsets of naive and memory T lymphocytes with different homing and effector capacities (45-47). We have recently used the

expression of CCR7 as a tool to delineate the lineage differentiation pattern of memory CD8+ T lymphocytes and to characterize the composition of the memory CD8+ T cell pool *in vivo* in antiviral immune responses. Ex vivo analysis identified four subsets of HIV-specific CD8+ T lymphocytes based on the expression of CCR7 and CD45RA antigens. The distribution of the different subsets of HIV-specific memory CD8+ T lymphocytes was then investigated in subjects containing both HIV- and cytomegalovirus (CMV)-specific CD8+ T cells. This analysis showed that the HIV-specific CD8+ memory T cell pool was predominantly (70%) composed of pre-terminally differentiated CD45RA-CCR7- cells whereas the CMV-specific memory cells pool consisted mostly (70%) of terminally differentiated CD45RA+CCR7- cells. These results demonstrate a selective impairment of the differentiation of HIV-specific memory CD8+ T cells during HIV infection.

A note of thanks

First of all, I want to tank the Foundation Council to award me with this prestigious prize. Switzerland has had a major impact in the development of my research and medical careers. The years spent at the Ludwig Institute for Cancer Research in Lausanne have influenced all the subsequent my research. If I can synthesize in few words, the goal of my research has been to transfer the concepts of fundamental immunology to the medical field in order to delineate the complex regulation of the immune response in virus infections. I want to take also the opportunity to thank a number of persons who have helped me over the past fifteen years. Alessandro and Lorenzo Moretta, who have been my mentors during the initial years of my research career in Lausanne, Anthony S. Fauci for his support and advice during the years spent at the National Institute of Allergy and Infectious diseases, National Institutes of Health, Bethesda, USA, and Jean-Charles Cerottini, Heidi Diggelman, and Michel P. Glauser for sponsoring my return in Switzerland and for the continuous support over the past four years. Finally, I want to thank the many collegues whose help, suggestions, discussion, confrontation and criticism have been essential to my work.

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LAUDATIO FOR DOCTOR BRIAN A. HEMMINGS

Professor Emmanuel Van Obberghen

I first made the acquaintance of Brian A. Hemmings in the late 1970's in the US. We were both working at the National Institutes of Health in Bethesda. We got to know each other because we attended a FAES sponsored social club just off the NIH campus. This club was mainly a meeting place for NIH laboratory heads and European postdoctorate (or Fogarty) fellows. The interactions at this club fostered many friendships and collaborations at the NIH, and after. At the time we did not initiate any collaborations - but we did attend many of the same parties! Our scientific interests were quite different at the time I was working in the laboratory of Jesse Roth on insulin action while Brian was in the laboratory of Earl Stadtman making inroads into regulation of metabolism by phosphorylation mediated by protein kinases and protein phosphatases. His work focused on yeast as a model system for protein phosphorylation which, at the time, was not appreciated by many. Of course, this dramatically changed a couple of years later when Paul Nurse discovered that the cell cycle regulatory gene cdc2 was a protein kinase and that it is highly conserved from yeast to man. Incidentally, Brian and Paul were Ph.D. students together in Norwich, England in the laboratory of the late Tony Sims.

At the end of 1979 Brian left NIH for what was to become quite a dramatic career change. He parted from NIH and yeast to work in the laboratory of Prof. Philip Cohen in Dundee where he took his first step into the world of studying the mechanism of insulin action, and signal transduction in general. During this period he was first to purify an obscure protein kinase, termed Glycogen Synthase Kinase-3 (GSK-3). GSK-3 activity was initially attributed by Josef Goris in Belgium to a protein that would activate a protein phosphatase, and by Philip Cohen in Dundee as a kinase that phosphorylated glycogen synthase. Subsequent experiments carried by Brian revealed that GSK-3 activated the protein

phosphatase by phosphorylating a regulatory subunit of the phosphatase. This was quite a paradigm change - kinases activating phosphatases - but this mechanism has recently been established for many different phosphatases whether they are serine/threonine, tyrosine or dual specific. After leaving Dundee, Brian moved to the Friedrich Miescher Institute in Basel (with a short stop-over in New York). At the FMI he started to develop his own research programs in the areas of signal transduction. During this initial period from 1984 to 1990 he participated in the cloning of many components of the major protein signaling pathway involving cAMP signaling. The first projects he tackled involved the cAMP-dependent protein kinase (PKA) and protein phosphatase 2A (PP2A). (Insulin signaling was to take a back seat for a few years). This was a very exciting period that led to the realization that all proteins were from gene families. PKA and PP2A were both complex and full genetic characterization involved in the purification of multiple protein complexes and cloning of numerous genes. PKA was essentially his tutor in terms of learning the techniques of molecular biology (it also led to the isolation of the first cDNA encoding PKB).

The other major contributions from the PKA era involved the realization that this kinase could be regulated by proteolysis - a regulatory mechanism not normally associated with protein kinases. Also, cell lines were developed that enabled elucidation of some of the steps in cAMP signaling pathway involved in regulating gene expression.

Towards the end of the 1980's Brian turned his attention to the problem of protein dephosphorylation. This work was largely carried out in collaboration with the late Stuart Stone and Jan Hofsteenge at the FMI, and Josef Goris at the Catholic University in Leuven, Belgium. Essentially they molecularly dissected the different complexes collectively known as PP2A. Protein complexes of PP2A were purified in Belgium and then sequenced and the corresponding genes cloned in Basel. This led to the isolation of several trimeric holoenzymes complexes of PP2A that are encoded by 5 gene families producing 16 different proteins. Following this intense period of cloning, these tools were used to discover more about the role of PP2A in Drosophila and mammalian cells. This era produced a series of significant papers in

many distinguished journals. Initially this work was considered his major scientific accomplishment. This basic work on the PP2A has formed the basis for much of our current understanding of PP2A.

The major conclusion from this work is that PP2A is implicated in all cellular processes. This is apparently due to the fact that PP2A is a negative regulator of more than 50 protein kinases implicated in all of the major signaling pathways. Not only does PP2A regulate the kinases in the signaling pathways but it also dephosphorylates the targets of many of the signaling pathways. This work still continues in his laboratory where they are trying to understand signaling pathways that modulate PP2A activity. Insights in this area could have significant importance to our understanding of the development of insulin resistance.

Due to some chance events a novel gene isolated during the PKA era was reactivated in the laboratory following the arrival of an Argentinean student, Fernando Pertossi, in the laboratory. This was the start of the PKB/Akt era. Brian's group was the first to clone the cDNA encoding PKB and characterize the kinase in 1991. This study was part of the era of homology cloning quickly followed by the introduction of PCR techniques. This led to the idea that the mammalian genome could encode around 1000 protein kinases. The recent completion of the sequencing of the human genome has confirmed the 1001 idea.

Following on the footsteps of PKB cloning, the groups of Staal and Tsichlis in the US working on a completely different topic - a rare transforming oncogene form a rodent T-cell lymphoma - dramatically intervened. The oncogene encoded by the Akt8 retrovirus was cloned and found to be the mouse homologue of PKB/Akt that was fused to the viral gag gene. Attachment of the gag sequence was sufficient to confer transforming properties to PKB/Akt. Further studies revealed that PKB/Akt is overexpressed in certain human cancers. This early work obviously suggested that PKB/Akt could be a significant, major player in the emerging signaling pathways. A very significant contribution that emerged from this work was the discovery of Pleckstrin homology (PH) domains. In collaboration with Richard Haslam in Canada, Brian published a short note in Nature (1993) regarding the

discovery of this 110 amino acid domain. PKB was the second protein to be identified with this module. It is now appreciated that approximately 150 different proteins have such a domains. Fiscke and colleagues made a ground breaking discovery shortly after, they found that PH domains from several proteins bind phospholipids with high affinity. It was quickly appreciated that these modules could function as membrane adaptors.

The next major advance came simultaneously from several laboratories. In short PKB/Akt could be activated following stimulation of cells by mitogens, growth factors and insulin. Thus, after a 10 year hiatus Brian's group returned to the field of insulin action with this seminal discovery regarding PKB regulation. In collaboration with a Ph.D. student, Mirjana Andjelkovic, he found that PKB could be dramatically activated by vanadate (an insulin mimetic) and insulin itself. The common elements in these regulatory events were the tyrosine kinase receptors that following agonist stimulation recruited the lipid kinases (PI3 kinase) that phosphorylated inositol phospholipids on the D3 position. This reaction produces PIP3, a signaling second messenger of considerable importance. Thus, the PH of PKB/Akt domain could function as an agonist inducible membrane adaptor important in the recruitment of PKB/Akt to the membrane. This is one of the key steps in PKB/Akt regulation.

In collaboration with Dario Alessi and Philip Cohen two major advances were made that had a significant impact on the insulin signaling field. During a visit to Dundee a collaboration was set up at the suggestion of Brian to investigate the identity of a novel insulin stimulated protein kinase. This study rapidly revealed that PKB/Akt was the insulin-activated kinase partially purified by Cohen and colleagues. Further they found that PKB/Akt phosphorylated and inactivated GSK-3, the key regulatory enzyme involved in the control of glycogen synthase and therefore glucose and glycogen metabolism. These results were published in Nature at the end of 1995 and ushered in a new era in the study of insulin action. This collaboration between the Hemmings and Cohen laboratories resulted in the delineation of the mechanism of activation of PKB/Akt by reversible phosphorylation.

Following the discovery of PKB regulation by insulin I got to know Brian for the second time in my life. We initiated collaboration on some aspects of insulin action in 1995. During a visit to Nice we realized that we had both been at NIH at the same time. By cross checking names we discovered that our paths had crossed nearly 20 years previously. (The key was the gold Beetle I drove in Bethesda).

Numerous significant advances have been made following these early studies. Since 1995 approximately 1000 papers have been published on the role of PKB/Akt. Parallel studies led to the realization that PKB/Akt is a major player in cell survival and the regulation of apoptosis. This has ultimately fostered the appreciation that PKB is a significant player in insulin action, cell survival and cancer. The pioneering studies by the Hemmings group in Basel, which made many seminal discoveries, has contributed immensely to these areas of research. As Brian once remarked in a Science article «the orphan kinase is now on center stage!»

DER AKADEMISCHE RAT
VERLEIHT

HERRN DOKTOR

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GEBOREN 1955 IN MANCHESTER/GREAT BRITAIN (ENGLAND)
FÜR HERVORRAGENDE LEISTUNGEN AM
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AUF DEM GEBIET DES ZELLSIGNALLING

ZÜRICH, 14. NOVEMBER 2001

FÜR DEN SENATSRAT

DER PRÄSIDENT

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Dr. Brian Hemmings

CURRICULUM VITAE

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1969 – 1972	B.Sc.(Hons) University of Nottingham, Nottingham, UK.
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Research Posts:

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1977 – 1980	Fogarty Visiting Fellow in the Laboratory of Dr Earl R. Stadtman, Laboratory of Biochemistry, National Institutes of Health, National Heart, Lung and Blood Institute, Bethesda, Maryland 20205, USA.
1980 – 1982	M.R.C. Research Fellow in the Laboratory of Professor Philip Cohen, Department of Biochemistry, Medical Sciences Institute, The University of Dundee, Dundee, DD1 4HN, Scotland.

1982 – 1983	Research Associate of the FMI in the Laboratory of Professor Edward Reich, The Rockefeller University, 1230 York Avenue, New York, NY 10021, USA.
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Membership of Professional Societies:

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The American Society for Biological Chemists (1983)
The Schweizerische Gesellschaft für Biochemie (1985)
The British Society for Developmental Biology (1992)
The British Society for Cell Biology (1992)
American Diabetes Association (1999)

Editorships:

Editorial advisory board of the Biochemical Journal (1986 – 1989)
Advances in Protein Phosphatases (1992 – present)
Member of the editorial board of the Journal of Biological Chemistry (1999 – present)

Reviewing:

On an ad hoc basis for Nature, Science, EMBO and Current Biology

Awards:

Chairman of EMBO Workshop on Protein Phosphatases (1995 – 1996), St. Moritz
Elected Member of EMBO (1996)
Co-organizer EMBO-FEBS Workshop on Protein Phosphatases and Protein Dephosphorylation (1997), Oxford

Member of the evaluation committee for the Göran Gustafsson Foundation (The Royal Swedish Academy of Sciences) for academic year 1998/1999
Delivered the Philip Godfrey Memorial Lecture of the Biochemical Society Meeting, Glasgow (1999)
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Visiting Professor (Bijzonder gasthoogleraar) at Faculty of Medicine, Katholieke Universiteit, Leuven, Belgium (2000)
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PROTEIN KINASE B (PKB/AKT) – A COMMON ELEMENT IN MULTIPLE SIGNALING PATHWAYS INVOLVED IN INSULIN SIGNALING, CELL SURVIVAL AND CANCER

Brian A. Hemmings

Introduction

All aspects of cell function depend on external signaling molecules in the form of growth factors, hormones or neurotransmitters or as proteins anchored to the surface of a neighbouring cell or the extracellular matrix. Signal transduction pathways function by coordinated activation or inactivation of proteins, within the cell membrane, cytoplasm or nucleus. Activation of a signal pathway usually involves binding of a ligand – hormones, mitogens, growth factors or neutrotransmitters – to its cognate receptor followed by phosphorylation or dephosphorylation of a target protein on tyrosine, serine or threonine residues by the appropriate downstream protein kinases or phosphatases. The phosphorylation state of a target protein or enzyme depends upon the relative activities of protein kinases and phosphatases in the pathway. Modules of protein kinases control cellular processes. This discovery - perhaps the most important in signal transduction during the past 10 years – is typified by growth factor stimulation of the Ras-Raf-MAP kinase module (1-3). One of the many initial events occuring after growth factors bind their cognate growth factor receptor tyrosine kinases is the recruitment and activation of the phosphoinositide 3-kinases (PI 3-kinases). Inositol lipids phosphorylated at the D3 position by PI3-kinases act as second messengers somewhat analogous to cAMP and Ca^{2+} (4,5).

The receptors specific for insulin and insulin-like growth factor-1 (IGF-1) also signal by inducing the recruitment of PI3-kinase (reviewed in 6). However, this activation mechanism is unique in the use of an adapter molecule, termed insulin receptor substrate (IRS) that bridges the interaction of insulin receptors and PI3-kinase. Following ligand binding, the receptor undergoes autophosphorylation on tyrosine residues initiating recruitment of the IRS adapter molecules and

tyrosine phosphorylation, recruitment of the lipid kinase followed by lipid phosphorylation and generation of the inositol lipid second messengers. That insulin induces profound effects on tyrosine phosphorylation was established relatively early. One of the major challenges was to find the protein kinase functioning as an «integrator» that decodes the lipid signals produced by the activation of PI3-kinase, and subsequently promotes the serine/threonine phosphorylation of intracellular proteins. The ultimate read-out from this pathway is recruitment of glucose transport proteins to plasma membranes and the subsequent uptake of glucose from the blood stream. Thus, for many years, researchers searched for the protein kinases activated by insulin.

My work on insulin signaling actually started in 1980. At that time, I was a post-doctorate fellow in the laboratory of Philip Cohen (Dundee) charged with the purification of a protein kinase with the potential to activate a protein phosphatase. This was considered to be a vital step in insulin action (another obsession at the time was the identification of the insulin second messenger). The kinase in this instance became known later as glycogen synthase kinase-3 (GSK-3) and was found to be acutely regulated by insulin. The molecule that emerged to link these disparate events is the topic of this lecture. The discovery of this particular protein kinase was tortuous, as is reflected by its different names – RAC, Akt and PKB. My laboratory was the first to clone the gene, which we termed RAC, but the kinase is now known as PKB/Akt, depending on which side of the Atlantic you are.

Protein kinase B is a serine/threonine kinase of the second messenger or AGC subfamily (7, 8). It is of considerable medical and pharmaceutical interest due to its connection with diabetes, cell survival and cancer (reviewed in 9, 10). PKB is a major downstream target of PI 3-kinase (11, 12), the lipid kinase involved in insulin signaling. The first evidence for the involvement of PKB in insulin signaling came from our work in 1995 with the laboratory of Philip Cohen (13). We demonstrated that PKB is responsible for the inactivation of glycogen synthase kinase 3 (GSK3) and may thus regulate glycogen synthesis in insulin-sensitive tissues. Since then, PKB has been linked to a number of insulin- and insulin-like growth factor-1 (IGF-1)-induced responses, inclu-

ding glucose uptake, protein synthesis, preadipose cell differentiation, and the regulation of gene expression (reviewed in 9, 10). In parallel, new functions of this kinase have emerged from the fields of cancer biology and neurobiology (reviewed in 9, 14 – 16).

Protein kinase B (PKB/Akt) – structure and regulation

The identification of two isoforms of PKB isoform (α and β) inaugurated a new sub-family of the second messenger-regulated kinases (7, 8, 17). Although both isoforms were found to be expressed ubiquitously, both their roles and their modes of regulation remained unclear. Work by Staal on the AKT8 retrovirus shed some light in this direction.

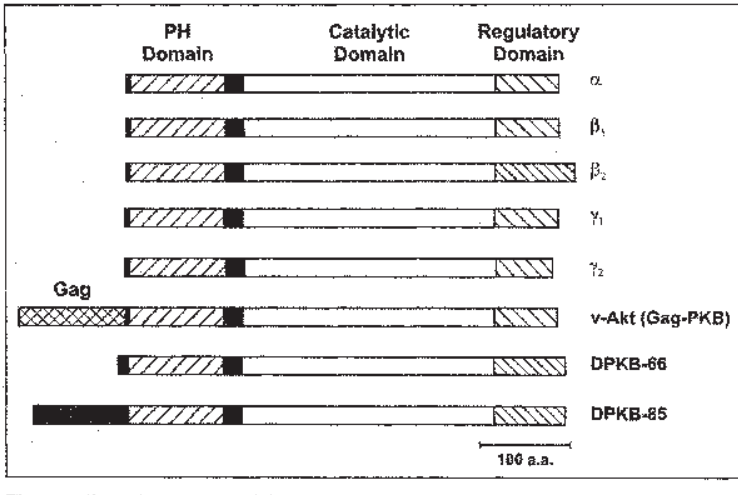

Figure 1. Domain structure of the mammalian and Drosphila PKB isoforms

AKT8 retrovirus induces T-cell leukemias and lymphomas in rodents and was isolated from a spontaneous lymphoma of an AKR mouse (18). The genome of the virus contains sequences of cellular origin designated v-Akt, and two homologues of the viral oncogene identified in the human genome were termed Akt1 (PKBα) and Akt2 (PKBβ) (19). Cloning of v-Akt led to the exciting discovery that it corresponds

to mouse PKBα or c-Akt (20). The third isoform PKBγ, cloned in 1995 from a rat cDNA library (21), has been connected recently to estrogen receptor-negative breast carcinomas in humans (22). Interestingly, the PKBγ human gene encodes two variants generated by alternative usage of 3' exons which results in different C-termini (23). All PKB isoforms have a common domain structure with a pleckstrin homology (PH) domain (24, 25) N-terminal to the central catalytic domain and a C-terminal regulatory region. The overall domain structure is preserved between the mammalian and Drosophila isoforms (Fig. 1).

Role of Phospholipids in PKB activation

PKB is activated by several growth and survival factors, including platelet-derived growth factor, epidermal growth factor, insulin, IGF-1, vascular endothelial growth, nerve growth factor, cytokines, chemokines, lysophosphatidic acid and serum (reviewed in 9, 10, 14 – 16). Several lines of evidence demonstrated that PKB activation is mediated through the action of PI 3-kinase. First, PKB activation is sensitive to the PI 3-kinase inhibitors (11, 12, 26) and LY 294002 (27). Second, PDGF receptor mutants which fail to activate PI 3-kinase are also unable to stimulate PKB (11, 12). Third, a dominant negative form of PI 3-kinase prevents PDGF- and insulin-induced activation of PKB (11, 28, 43). Fourth, constitutively active forms of PI 3-kinase stimulate PKB (29, 30, 31).

How does PI 3-kinase promote PKB activation? The crucial role in this process is performed by 3-phosphoinositides. This class of phospholipid second messenger is produced by the action of PI 3-kinase, which synthesises phosphatidylinositol 3,4,5-bisphosphate [PtdIns(3,4,5)P_3] (reviewed in 4). The latter phospholipid is a substrate of inositol polyphosphate 5-phosphatase, which modifies it to phosphatidylinositol 3,4-bisphosphate [PtdIns(3,4)P_2], thus changing the ratio between the two classes of 3-phosphorylated phosphoinositides (32). Both PtdIns (3, 4, 5)P_3 and PtdIns(3,4)P_2 act as second messengers able to recruit a number of target proteins to the membrane (4). The lipid signal is «switched off» by the tumor suppressor MMAC/PTEN, a phosphatidy-

linositol phosphatase which removes the phosphate from the 3' position of the inositol ring in PtdIns(3,4,5)P_3. (33 – 35).

The major targets of 3-phosphoinositides are PH domain-containing proteins (36, 37). Such signaling modules of about 100 amino acids have been identified in over 150 signaling or cytoskeletal proteins, most of them able to associate with the cell membrane (25, 38). Conservation between different PH domains at the primary structure level is low (reviewed in 38). However, the three-dimensional structures of PH domains from β-spectrin, pleckstrin, dynamin and phospholipase Cδ1 revealed remarkable conservation. It was anticipated from the structure that this signaling module would bind lipophilic molecules and this was first demonstrated for PtdIns(4,5)P_2 and isolated PH domains of pleckstrin, RasGAP, and protein kinases Tsk and β-Ark (39). As PKB was found to be a target of PI 3-kinase, it was not difficult to foresee the binding of 3-phosphoinositides to its PH domain. Indeed, the PH domain of PKB binds PtdIns(3,4,5)P_3 and PtdIns(3,4)P_2 *in vitro* with similar submicromolar affinities (40, 41). PKB molecules carrying mutations in the PH domain do not respond to PDGF stimulation and are not activated by PtdIns(3,4)P_2 (12, 42). The high-affinity interaction between the PH domain and 3-phosphoinositides is sufficient for recruitment of PKB to the site of their production *in vivo*. Thus, growth factor binding to their cognate receptors results in PI 3-kinase activation and production of the lipid second messenger at the plasma membrane, which represents the «switch-on» mechanism for PKB activation. Translocation of the kinase to the plasma membrane has been demonstrated following IGF-1 stimulation and is sensitive to the PI 3-kinase inhibitor wortmannin, which requires the PH domain (43). This step is needed for priming the kinase for activation by phosphorylation (43). Targeting of PKB to the plasma membrane results in a constitutively active kinase (43) and reduces the requirement for PI 3-kinase in PKB activation. This also offers an explanation for the oncogenic potential of v-Akt, a chimera of viral gag protein and PKB, in which the N-terminal myristic acid group alters the subcellular localisation and makes the kinase constitutively active (43, 44).

Role of phosphorylation in PKB activation

The results of stimulating PKB activity by treating cells with phosphatase inhibitors provided the clue that phosphorylation is a mechanism for regulating activity (28). Furthermore, the kinase isolated from stimulated cells could be inactivated *in vitro* by treatment with protein phosphatase 2A (PP2A). PKB was found to be phosphorylated in unstimulated cells and phosphorylation levels increased following stimulation by PDGF or pervanadate (11, 28). While PDGF augmented Ser phosphorylation (11), the potent PKB activator pervanadate (an insulin mimetic) induced phosphorylation on both serine and threonine residues (28). Mapping the phosphorylation sites of PKBα from human

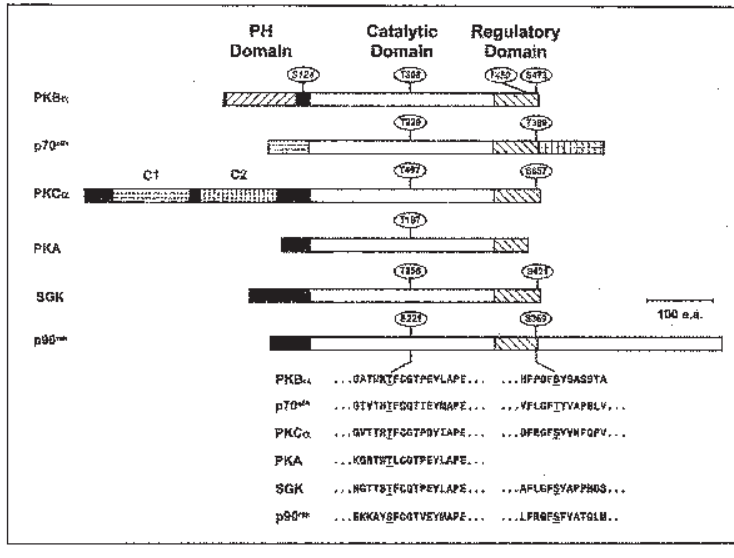

Figure 2. Constitutive and regulatory phosphorylation sites of protein kinase B, and other related members of the AGC family of kinases.

embryo kidney (HEK) 293 cells revealed that the kinase is constitutively phosphorylated on Ser124, in the hinge region between the PH and catalytic domains, and on Thr450, in the C-terminal region in unstimulated cells (45). Concomitant with insulin- or IGF-1-induced

PKB activation, two additional sites appeared: Thr308/Thr309 in the activation loop of the catalytic domain and Ser473/Ser474 in the C-terminus of the α and β isoforms, respectively, (45, 46). Like PKB activation, phosphorylation of the two induced sites was found to be sensitive to PI 3-kinase inhibitors (45, 46). Mutation of either site to Ala abolished insulin-induced PKB activation. Conversion of both sites to Asp (PKB-T308D/S473D) to mimic phosphorylation yielded a constitutively active form of the kinase resistant to wortmannin, which could not be activated further (45). Constitutive and regulatory phosphorylation sites on PKB are depicted in Figure 2.

The mechanism of PKB phosphorylation at Thr308 and Ser473 was quickly elucidated. Several lines of evidence excluded PKB itself as the enzyme responsible for this phosphorylation. Firstly, only a restricted amount of overexpressed wild-type PKB is activated following stimulation of the cells, implying the existence of an upstream limiting factor (28). Secondly, the use of kinase-defective PKB excluded autophosphorylation as a regulatory mechanism (26, 28). Thirdly, the sequences flanking these regulatory residues are not compatible with the consensus phosphorylation sequence for PKB. The different con-

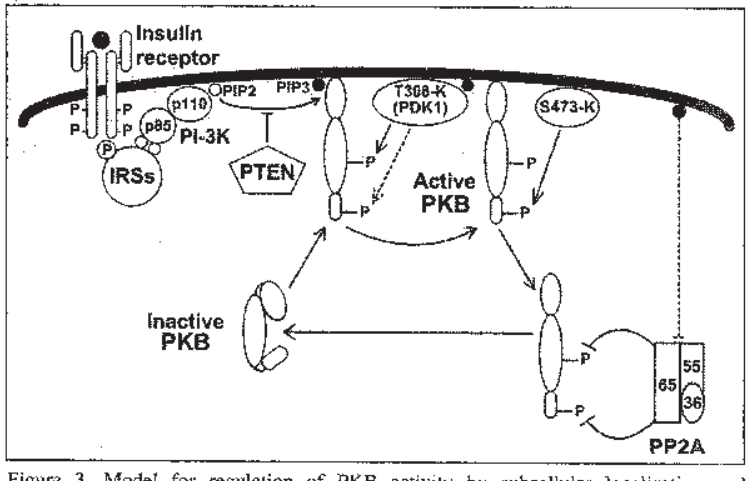

Figure 3. Model for regulation of PKB activity by subcellular localization and phosphorylation by upstream kinases.

texts of the Thr308 and Ser473 phosphorylation sites pointed towards the existence of two upstream kinases. The fact that PKB becomes phosphorylated on these residues simply by membrane targeting without cell stimulation implies that the upstream kinases are already membrane-associated in unstimulated cells and may themselves be regulated by PI 3-kinase (43, 52). A current model depicting some of the major steps in PKB activation is shown in Figure 3.

The regulation site Thr308 is located between subdomains VII and VIII of the catalytic domain of PKBα, between the conserved DFG-APE motifs (47). The crystal structures of PKA, cyclin-dependent kinase 2, MAPK and Ca^{2}+-calmodulin-dependent protein kinase (CaMK-I) identified this region as the activation- or T-loop (48). The first PKB upstream kinase was isolated independently by two research groups using its unique property to phosphorylate PKB on Thr308 in the presence of 3-phosphoinositides (49, 50). Because of the phospholipid requirement for phosphorylating PKB on Thr308, the kinase was named 3-phosphoinositide-dependent protein kinase 1 (PDK1) (51). Cloning of PDK1 revealed that it contains a PH domain at the C-terminal region, which binds 3-phosphoinositides (51), similar to PKB. Interestingly, removal of the PH domain of PKB ablated the phospholipid demand for phosphorylation by PDK1 (49-51). This may be explained by the fact that the PH domain in the inactive PKB sterically hinders the phosphorylation site in the catalytic domain. Phospholipid binding to the PH domain opens up the kinase conformation, allowing the upstream kinase access to its substrate. The inhibitory effect of the PH domain has been confirmed by *in vivo* studies. Its removal has a stimulatory effect on PKB activity and it facilitates activation by PDK1 *in vivo* (52).

The isolation and cloning of PDK1 marked a new era in the regulation of second messenger-regulated kinases. Striking similarities between the regulatory phosphorylation sites in the activation loop of PKB and those identified in other second messenger-regulated kinases opened up the possibility that PDK1 can also phosphorylate the homologous site in the catalytic domain of other kinases (Figure 2). This has been confirmed in several instances. For example, PDK1 readily phosphorylates a site in the catalytic domain of p70 S6K both *in vivo* and *in vitro*,

when the main requirement – free access to the substrate – is fulfilled (53). In contrast to PKB, where this is achieved by acting on the N-terminal PH domain, p70 S6K requires prior hierarchical phosphorylation C-terminal to the catalytic domain. PKA and PKC are also substrates for PDK1. Two PKC isoforms have been reported to be phosphorylated both *in vitro* and *in vivo* by PDK1 (54). Likewise PDK1 can phosphorylate Thr197 or PKA, resulting in activation of the catalytic subunit *in vitro* (55). Therefore, PDK1, or a related kinase, is likely to be the PKA kinase *in vivo*.

Serum and glucocorticoid-inducible kinase (SGK) appears to be another target of PDK1. This transcriptionally-regulated kinase is activated by insulin, IGF-1, peroxide and pervanadate in a PI 3-kinase-dependent manner, which makes it similar to PKB (56, 57). Coexpression of PDK1 activates SGK in vivo and PDK1 was found to interact with the catalytic domain of SGK (57). Despite a high degree of similarity to PKB (57% identity in the catalytic domain) and conservation of the phosphorylation sites, SGK does not possess a PH domain; it is still not clear how the signal from PI 3-kinase is translated into phosphorylation. Based on these properties, SGK represents a new kinase on the horizon of insulin signaling. p90RSK is a kinase with two catalytic domains. The N-terminal kinase domain has all the properties of second messenger-regulated kinases and is involved in substrate phosphorylation. Its activation by phosphorylation on Ser227 in the activation loop is carried out by PDK1 both *in vitro* and *in vivo* (58).

The second PKB regulatory site Ser473, which is surrounded by aromatic, hydrophobic residues, is homologous to the major rapamycin-sensitive site of p70 S6K, the Thr389 located C-terminal to the catalytic domain (59). A similar consensus sequence is present in conventional and novel PKC isoforms, p90 RSK, SGK and Ndr (Figure 3) (57, 58, 60). While phosphorylation of Ser473 has been firmly demonstrated in PKB regulation, the identity of the kinase has not been clearly established. Several candidates listed below have been implicated directly or indirectly in phosphorylation of this site but in my opinion, none of them appears to fulfil the criteria for the authentic *in vivo* kinase.

The «classical» biochemical approach, where the C-terminal peptide of PKB including the phosphorylation site was used as a substrate to purify kinase activity, resulted in the isolation of MAP kinase-activated protein kinase 2 (27). This enzyme is a component of the stress- and cytokine-activated signaling pathway. Although MAPKAP-K2 can phosphorylate PKB on Ser473 and activate it *in vitro*, it does not appear to be the *in vivo* upstream kinase. First, it is activated by cellular stresses which do not activate PKB and, second, inhibition of the signaling pathway on which MAPKAP-K2 lies does not block PKB activation *in vivo*.

The integrin linked kinase (ILK) is activated by insulin and fibronectin *in vivo* in a PI 3-kinase-dependent fashion and can also be stimulated by 3-phosphoinositides *in vitro* (61). ILK does not contain a canonical PH domain but a putative phospholipid-binding motif has been identified, with a sequence corresponding to subdomains I and II of PH domains from several signaling molecules. ILK phosphorylates PKB on Ser473 *in vitro* and coexpression of a kinase-inactive form suppresses PKB activation and Ser473 phosphorylation *in vivo*. ILK overexpression in cells promotes adhesion-independent growth and survival and cell cycle progression (62) and induces tumors in nude mice (63). Its anti-apoptotic role and oncogenic potential makes it similar to PKB and it is thus a likely candidate for Ser473 kinase. However, we were not able to detect changes in Ser473 phosphorylation in PKB upon coexpressing ILK in either unstimulated or insulin-stimulated HEK 293 cells (52). Thus, the exact role of ILK in PKB regulation remains open.

PKCζ has been shown to phosphorylate the site homologous to Ser473 in PKCα and PKCδ in the C-terminal variable 5 (V5) domain (64). Serum-induced phosphorylation at this site is sensitive to PI 3-kinase inhibition and to the immunosupressant rapamycin, similar to p70 S6K. PKCζ itself possesses the hydrophobic motif in the V5 domain found in conventional and novel PKC isotypes, but with an acidic residue instead of phosphorylatable Ser (64). Coexpression of constitutively active PKCζ overcomes LY294002 and rapamycin sensitivity of PKCδ phosphorylation on Ser661, whereas a dominant-negative mutant pre-

vents phosphorylation at the same site. PKCζ is itself a downstream target of PI 3-kinase and has been implicated in insulin signaling (65, 66), which opens up the possibility that this kinase also acts on PKB. A link between the two kinases was made by Konishi et al., (21), who identified PKCζ as a protein interacting with the PH domain of PKB. However, the activation of PKB by insulin, PDGF, or serum is not sensitive to the inhibitory effects of rapamycin (11, 13, 28), as well as serum-induced phosphorylation on Ser473 (Andjelkovic and Hemmings, unpublished observation). Therefore, the role of PKCζ in PKB regulation still remains to be clarified.

Downstream signaling and the biological function of PKB

In order to understand the physiological function of PKB, it was important to identify authentic substrates. Since 1995 the range of PKB-regulated functions has dramatically increased. This is in part due to the rea-

PKB substrate proteins

Proteins	PKB phosphorylation sites	Species	Effects of phosphorylation
Bad	107RSRHSSY113 130RGRSRSA137	human	blocks Bad-induced apoptosis
C-Raf	259RQRSTST265	human	inhibits C-Raf activity
B-Raf	359RDRSSSA365 427RERKSSS429	human	inhibits B-Raf activity
BRCA-1	308KKRRPTS315	human	interferes with the nuclear localization of BRCA-1 ?
Caspase-9	191RRRFSSL197	human	inactivates Caspase-9 activity
CREB	129LSRRPSY134	human	increases its association with CBP and p130
eNOS	1172RIRHSSY1179	human	activates eNOS activity and leads NO production
FKHRL1	246RRRAASM252	human	inhibits transcription activity of FKHRL1
GSK-3 α	16RARTSSF22	human	inactivates GSK-3 activity
GSK-3 β	9RPRTSSF15	human	
I-kB kinase α	176REKLGTG184	human	activates transcription activity of NF-kB
IRS-1	297RSRTESP303	mouse	protec ts IRS-1 from the action of PYPase
mTOR	2443RTRTDSY2449	mouse	inhibits mTOR activity
PDE-3B	268RPRRRSS274	mouse	inactivates PDE-3B activity
PFK-2	466RMRRNSF472 478RPRNTSV484	bovine	activates PFK-2 activity
Rac1	66RIRPLSY72	human	inhibits Rac1-GTP binding
hTERT	819RIRGKSY825	human	enhances telomerase activity

lization that the protein kinase GSK-3 is a major target of PKB, and because a detailed substrate-specificity study was undertaken that defined the consensus PKB phosphorylation site as Arg-X-Arg-Y-Z-Ser/Thr-Hyd, where X is any amino acid, Y and Z are small residues other than Gly and Hyd is a bulky hydrophobic residue (67). Database searches identified a large number of proteins with this consensus site and played a very significant role in the identification of a vast array of substrates involved in many different cellular processes, such as regulation of glucose metabolism in insulin-response tissues, cell survival, transcription, protein synthesis, cell cycle progression and cell transformation. A current list of identified PKB substrates is shown in Table 1.

Insulin signaling

PKB can regulate glucose metabolism at several levels and, therefore, has had a major impact on our understanding of insulin signaling (see Figure 4). The realization that PKB activation is PI 3-kinase dependent

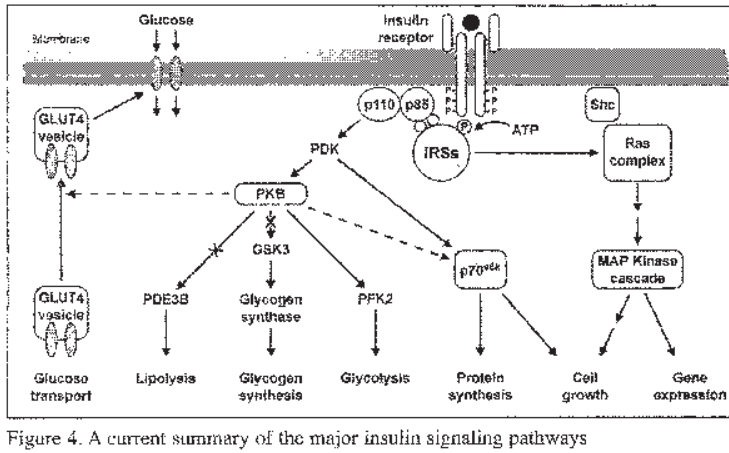

Figure 4. A current summary of the major insulin signaling pathways

was a major breakthrough indicating that this kinase is a major player in signal transduction. The second major advance was the discovery that PKB regulated GSK3 (the first physiological substrate) in an insu-

lin-dependent manner (13). This information essentially linked insulin receptor activation, through a protein kinase cascade, to the regulation of glycogen metabolism. Serine21 in GSK3α and Serine9 in GSK3β are phosphorylated by PKB and this serves to negatively regulate GSK3 activity and to maintain glycogen synthase in a dephosphorylated and active state (13). Thus, insulin stimulation of glycogen synthesis is mediated through the inhibition of GSK3 coupled with simultaneous activation of the relevant glycogen synthase phosphatase. The identification of PDK1 as a regulator of several protein kinases activated by insulin provided a further layer of complexity to insulin signaling (see Figure 5).

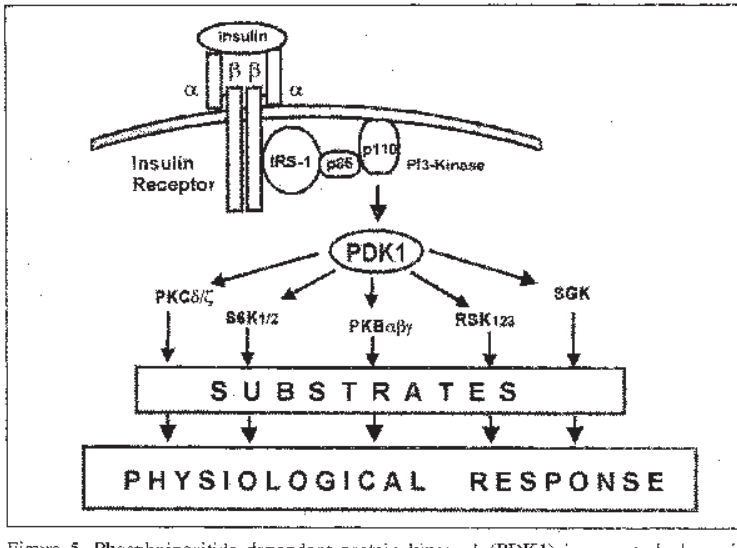

Figure 5. Phosphoinositide dependent protein kinase 1 (PDK1) is a central player in insulin signaling

In parallel to these observations, it was reported that insulin-mediated activation of PKB also promoted glucose uptake by affecting the GLUT1, GLUT3 and GLUT4 glucose transporters (68, 69). Apparently, activated PKB induces the expression of GLUT1 and 3 and the translocation of GLUT4 to the plasma membrane. However, these results are somewhat controversial and we still need a detailed mole-

cular description of the mechanism for stimulating membrane recruitment. Recently, GLUT4-containing vesicles were found to have a specific association with PKBβ (70). Direct phosphorylation of the transporter is still a possible mechanism. Furthermore PKB has been implicated in the regulation of two further classical insulin responses: 6-phosphofructose-2-kinase (PFK-2) and phosphodiesterase 3B (PDE3B). Deprez et al. (71) proposed that PKB mediates the insulin-induced activation of heart PFK-2 by phosphorylating Ser466 and Ser483. Insulin inhibits triglyceride hydrolysis apparently by activating PDE3B through phosphorylation of Ser302. The data so far available indicate that PKB phosphorylates the critical PDE regulatory site (72).

Cell survival

Considerable evidence has accumulated indicating that PKB plays a major role in growth factor-mediated cell survival and inhibition of apoptosis in response to several different stimuli (see Figure 6). PI 3-

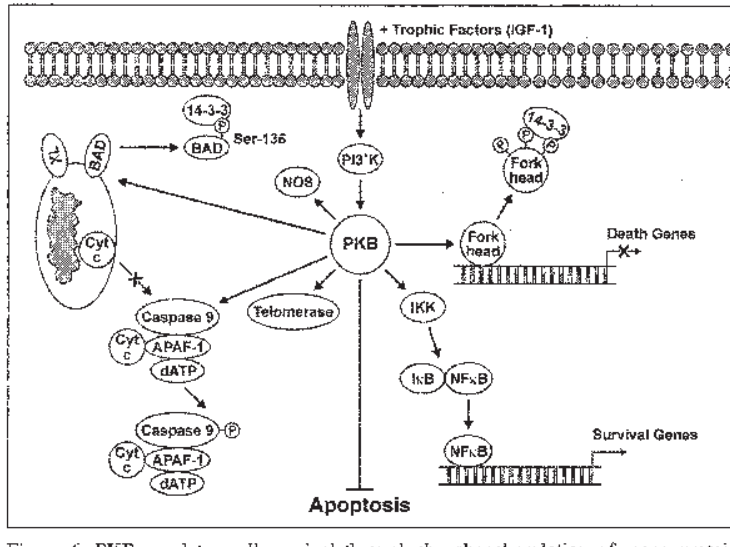

Figure 6. PKB regulates cell survival through the phosphorylation of many proteins involved in the regulation of apoptosis.

kinase was first implicated in the suppression of apoptosis in a study by Yao and Cooper (73). These results suggested that PKB is a critical regulator of cell survival. The idea was first tested in a study with cultured cerebellar granule cells that investigated the signaling molecules utilized by IGF-1 and PI 3-kinase to promote cell survival (74). The results revealed that constitutively active PKB promoted cell survival in the absence of IGF-1. Numerous studies have now shown that PKB blocks apoptosis induced by apoptotic stimuli, such as removal of growth factors, UV irradiation, matrix detachment, cell cycle arrest, DNA damage, and treatment of cells with anti-Fas antibody or TGFβ (reviewed in 14-16). Subsequently, targets were sought that were relevant to the survival-promoting effects of PKB. This was aided by work defining the substrate specificity of PKB (67). Many of the proteins of the apoptosis machinery contain PKB consensus sites (15). Interestingly we demonstrated that NGF activates all three isoforms of PKB in PC12 cells (75) but little attention has been paid to the role of the different isoforms in cell survival signaling.

One mechanism by which PKB may promote survival is through the inhibition of a component of the cell-death machinery. Among molecules central to the regulation of cell death in eukaryotes are members of the BCL-2 family of proteins (15). Several members of the BCL-2 family (BCL-2, BCL-XL, MCL-1, A1 and BAG-1) promote survival, whereas others (BCL-XS, BAD, BAX and BAK) promote cell death (15). BCL-2 family members homo-and hetero-dimerise and the balance between particular homo- and heterodimers is critical in the maintenance of cell survival or the induction of apoptosis. The fact that BAD is phosphorylated in vivo on a consensus PKB site (76) ushered in an era of research that dramatically changed our understanding of the regulation of cell death. It is now appreciated that PKB can regulate apoptosis at multiple levels. Several direct substrates, including BAD (76), caspase 9 (77), the Forkhead family of transcription factors (78-82) and the NF-kB regulator kinase (83 – 86), play critical roles in mediating cell-death.

Following survival factor treatment BAD is phosphorylated on two serine residues, Ser116 and Ser136, embedded in 14-3-3 consensus

binding sites causes BAD to dissociate from BCL-XL/BCL-2, and to associate with 14-3-3 proteins (87). BAD phosphorylation is promoted by a PI 3-kinase/PKB pathway upon stimulation by PDGF or IGF-1 (76). PKB is a potent Ser136 kinase *in vitro*, and *in vivo*. Recent results show that PKB primarily triggers BAD phosphorylation at Ser136 and that phosphorylation at this site is sufficient to promote survival. More recent data suggest that several protein kinases target the multiple sites on BAD and contribute to cell survival (15, 88). The degree of importance of PKB in cell survival signaling was expanded by the observation that PKB phosphorylated and inactivated caspase 9 (77). This result explains how growth factors can block cell death subsequent to increases in cytoplasmic cytochrome C (89). Increases in the concentration of cytochrome C in the cytoplasm result in the nucleation of a multienzyme complex, the apoptosome, comprising Apaf-1, cytochrome C, dATP, and the aspartyl-directed protease caspase 9. Inactivation of caspase 9 by PKB blocks the activation of this complex, even in conditions promoting cytochrome C release from the mitochondria. Genetic studies by the groups of Rufkun and Kenyon, using the worm C.elegans to study the control of diapause formation and longevity provided critical data identifiying DAF-16, a Forkhead family member, as a target of the PI 3-kinase/PKB pathway (90, 91). These observations, linked to the fact that many apoptotic stimuli also induce de novo gene expression as an essential part of the apoptotic program, prompted many laboratories to investigate whether the Forkhead transcription factors are phosphorylated by PKB (78-82). Mammalian cells produce three isoforms of these transcription factors (FKHR, FKHRL1 and AFX). Significantly, these three isoforms have been identified at the sites of chromosomal rearrangements in certain human tumors (92). PKB phosphorylates all members of the Forkhead family on several sites (78-82). Thus far, three sites have been mapped and the major site of phosphorylation by PKB is the second C-terminal site in the DNA-binding domain. Phosphorylation of Forkhead isoforms at each of the three sites is induced upon exposure to IGF-1. The fact that PKBα and β translocate (43) to the nucleus following stimulation maybe a critical component of this signaling mechanism, however, it has also been suggested that PKB phosphorylates these transcription factors in the cytoplasm.

Growth regulation and cancer

Following our report on the molecular cloning of PKB in 1991, the group of Tsichlis made the dramatic observation that the oncogene from the AKT8 retrovirus corresponds to the mouse PKBα (cAkt) fused to the viral gag protein (20). The AKT8 retrovirus was first isolated some 15 years earlier by Staal and colleagues, who proceeded to do some very insightful work without identifying or cloning the v-Akt gene (18, 19). This observation placed PKB in an elite, the proto-oncogene group of serine/threonine specific protein kinases, which includes c-Raf, c-Mos, Pim1, Cot1, Aurora 2, MEK and PKC epsilon (93). Since the mechanism of PKB activation has been investigated in considerable detail, it is now possible to rationalise why v-Akt is oncogenic. Attachment of the gag sequence introduces an N-terminal myristic acid that targets PKB to membrane (43, 44, 52). We demonstrated that this is sufficient to promote the phosphorylation of PKB on Thr308 and Ser473 and produce a constitutively active form of the kinase (43). This could have two possible effects: a) constitutive phosphorylation of substrates such as the Forkhead transcription factors, BAD, and possibly GSK3, thereby blocking apoptosis, or b) phosphorylation of as yet unidentified substrates capable of bringing about cell transformation.

The oncogenic potential of the PI 3-kinase/PKB pathway has been emphasised recently by the discovery that the catalytic subunit of PI 3-kinase is an oncogene (94), and the fact that the tumor suppressor gene PTEN is an upstream negative regulator of PKB (33-35). Significantly the avian sarcomavirus PI 3-kinase derived oncogene (v-p3k) is a fusion between the catalytic subunit of the lipid kinase and the viral gag gene (94). This AVI6 oncogene has the potential to induce haemangiosarcomas in chickens and transform chicken embryo fibroblasts. CEFs transformed by v-p3k have elevated PIP2 and PIP3 and enhanced PKB activity. A further connection between PI 3-kinase, PKB and cellular transformation can be found in the realization that Ras, a small GTP-binding protein mutated in many human cancers, is an activator of PI 3-kinase (95).

In the case of PTEN, its role in PKB was unsuspected. Initially isolated as a tumor suppressor gene mutated in many cancers, most impor-

tantly in the rare hereditary breast and thyrorid cancer predisposition syndrome, Cowden's disease, the PTEN gene was thought to encode a dual-specificity tyrosine phosphatase with preference for acid substrates (reviewed in 96). This led to the dramatic discovery that PTEN prefers phospholipids as substrates and specifically dephosphorylates the D3 position of the $PI(3,4)P_2$ and $PI(3,4,5)P_3$ products of PI 3-kinase (33, 34). It was recognised immediately that PTEN could function as a negative regulator of the PKB signaling pathway. Normally, PKB activity is low in the absence of growth factors. PTEN-deficient tumor cell lines, as well as immortalised fibroblasts and tumors derived from PTEN-deficient mice, show high levels of PKB phosphorylation. Furthermore, PTEN-/- fibroblasts are resistant to several anti-apoptotic stimuli, consistent with negative regulation of apoptosis by PKB. All these data clearly implicate PKB in the development of cancer. Further data to support this idea come from the study of PKB expression in human tumors. PKBα was found to be amplified in some human gastric adenocarcinomas (19), whereas PKBβ amplification was found in human ovarian carcinomas (97). Further examples of PKBβ amplification and/or overexpression were found in pancreatic carcinomas (98, 99). PKBγ has been found to be upregulated in estrogen receptor-deficient breast cancer and in androgen-independent prostate cancer cell lines (22).

Our recent work has revealed a new player in this complex signaling pathway (100). We have isolated a novel protein, CTMP, that appears to function as a tumor suppressor because it can negatively regulate PKB by inhibiting kinase activation. The growth of v-Akt tumors could be blocked or significantly inhibited by overexpressing CTMP. Furthermore, TCL-1, a proto-oncogene involved in prolymphocytic leukaemia, was found recently to stimulate PKB activity (101, 102). Here, a new paradigm appears to be emerging that involves a novel group of adaptor proteins that modulate PKB activation and activity.

Thus, a considerable body of information now directly or indirectly implicates PKB in the development of cancer. Our task in the future will be to develop therapeutics that can successfully ablate the kinase without significantly affecting glucose metabolism.

Concluding remarks

Over the past 3 years, our appreciation of PKB as a key component in several signaling pathways has increased dramatically. The nature of these pathways, which impinge on such diseases as diabetes, neurodegeneration and cancer, mean that PKB will remain the focus of intense research activity in the coming years. Obviously, there are many unanswered questions regarding PKB function. The unfolding of the PKB story, nonetheless, illustrates the need for basic research programmes focusing on fundamental mechanisms, which will eventually lead to a better understanding of cellular regulatory processes. Such an approach linked to the new techniques encompassed by genomics and proteomics, should provide us with the information and tools to develop therapeutics needed to manage some of the major diseases currently afflicting the western population.

We should also keep in mind that the current estimate of protein kinases in the human genome is around 2000 (a significant number at the start of the new millennium). Of the known, or intensely studied, protein kinases, probably less than 5 – 10% can be ascribed functions in a known signaling pathways. In other words, we still have to discover 90% of the signaling pathways encoded by the human genome and to delineate their functions. Despite the immense amount of work ahead, many of the major paradigms are now mapped out and these provide a firm framework to discover new signaling pathways.

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