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STIFTUNG PROFESSOR DR. MAX CLOËTTA

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Prof. Dr. Amalio Telenti
«Adaptation, co-evolution, and human susceptibility to HIV-1 infection»

Prof. Dr. Radek C. Skoda
«The control of normal and aberrant megakaryopoiesis
by thrombopoietin and its receptor, c-MPL»

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VORWORT

Prof. Max M. Burger
Präsident des Stiftungsrates

Im Rahmen des 30-jährigen Bestehens unserer Stiftung galt die letztjährige Preisverleihung ausnahmsweise einer Rückschau über das Erreichte. Diesmal ist es Zeit, sich nach einer derartigen Nabelschau wieder mit den Zukunftsproblemen der biomedizinischen Forschung zu beschäftigen. Bei der Abwägung unserer Forschungsstellengesuche wie auch bei der Preiszusprache stossen wir immer wieder auf das Problem, dass der Vergleich zwischen der grundlagenorientierten Forschung und der patientenorientierten klinischen Forschung auf Grund von bibliometrischen Unterlagen in den letzten Dezennien stets schwieriger geworden ist. Dasselbe gilt auch für andere Preiskomitees. So wurde und wird der Nobelpreis für Medizin und Physiologie und Medizin nur ganz selten für patientenbezogene klinische Forschung verliehen.

Das Problem ist nun aber keineswegs den Preiskomitees vorbehalten. Die Forschungsförderung der beiden Sparten der Biomedizin kämpft mit denselben Schwierigkeiten. So haben Mike Bishop und Hal Varmus kürzlich die Leitung grösserer medizinischer Zentren übernommen, der eine als Chancellor der University of California in San Fransisco, der andere als Direktor des Memorial-Sloan-Kettering-Krebszentrums in New York, und beide haben sich als eine der Hauptaufgaben die Förderung der patientenbezogenen klinischen Forschung auf ihre Fahnen geschrieben. Mike Bishop hat mir kürzlich anlässlich einer Evaluierung des Dana-Farber-Krebsinstituts der Harvard-Universität eingestanden, dass er zurzeit den grössten Teil seiner «administrativen Denkzeit» diesem Thema zuwende.

Vor wenigen Wochen kam die Stiftung der American Cancer Society (ACS) im Rahmen eines kleinen Think-Tank-Meetings zum Schluss, alles zu unternehmen, um junge klinische Forscher mit einem neuen Programm teil- oder zeitweise vom klinischen Routinebetrieb zu

befreien und ihnen damit mehr Forschungsfreiraum zur Verfügung zu stellen. Zusammen mit Andrew von Eschenbach, dem neuen Direktor des nationalen Krebsinstituts (USA) werden nun Aktionen – auch mit ACS-Geldern – in dieser Richtung unternommen.

Dass dasselbe Problem auch bei uns in der Schweiz vorhanden ist, steht ausser Zweifel. So kam auch der Wissenschafts- und Technologierat in einem Bericht vor zwei Jahren zum Schluss, dass die patientenorientierte klinische Forschung Mühe hat, mit der Qualität der biomedizinischen Grundlagenforschung mitzuhalten. Die Parameter sind aber teilweise doch verschieden, und andere Gründe, vor allem in der Schweiz, beeinträchtigen unsere internationale Konkurrenzfähigkeit, wie z. B. die begrenzte Anzahl von Patienten, die für eine aussagekräftige Studie über eine spezifische klinische Fragestellung rekrutiert werden müssten. Für jede forschungsfördernde Stiftung oder Organisation wäre es ein Einfaches, bei der Beurteilung der Qualität der Gesuche allein auf den «Impact», d. h. den Beachtungsgrad der Forschungsarbeiten, zurückzugreifen. Eine solche, rein bibliometrische Betrachtungsweise ist aber gerade in der Schweiz gefährlich, da die Schweiz mehrere biomedizinische Grundlagenfächer aufweist, die weltweit die Nummer eins (in Biologie und Biochemie sowie Immunologie) oder hinter den USA die Nummer zwei (in Molekularbiologie und Genetik) auf dem Rücken tragen. In einem Vergleich mit den klinischen Fächern könnten damit nur medizinische Grundlagengesuche gefördert und honoriert werden.

Die Stiftung Prof. Dr. Max Cloëtta hat deshalb schon kurz nach ihrer Startphase begonnen, ihren Preis in der Regel in einen eher klinisch orientierten und einen eher grundlagenforschungsorientierten Preis aufzuteilen. Auch bei den Forschungsstellengesuchen widmen wir den klinisch orientierten Gesuchen – wie beim Golf – ein Handicap, d. h., wenn immer möglich ein spezielles Augenmerk. Viele solche Anstrengungen, nicht zuletzt in Kreisen der klinischen Forschung in der Schweiz selber, haben die Leistungen in diesem Gebiet, die noch vor 19 Jahren in einer OECD-Studie recht kritisch beurteilt wurden, in den letzten Jahren neu auf den weltweit sehr beachtlichen 11. Platz in der CEST-Impact-Studie emporgehoben. Weitere Initiativen werden aber nötig, um den Schwung, der sicher auch direkt aus der medizinischen

Grundlagenforschung in der Schweiz kommt, für die klinische Forschung auszunützen und den Verschiebungen von klinischen Forschungsstellen auf Routinekliniksstellen entgegenzuwirken. Laut Daniel Scheidegger sind z. B. Bestrebungen im Gange, auch bei den nationalen Forschungskompetenzzentren (NCCR), die historisch bedingt mehr auf biomedizinische Grundlagenforschung als auf klinische patientenorientierte Forschung ausgerichtet sind, da und dort etwas stärker auf klinisch relevante Fragestellungen auszurichten.

Noch direkter wird die klinische Forschung von einer Initiative des Nationalfonds profitieren. Kürzlich wurde dort eine Untergruppe für patientenorientierte klinische Forschung (PaKliF) mit eigenem Budget eingeführt. Damit werden grosse Kohorten-Studien über gewisse Krankheiten durchführbar. Dies zum Teil nach dem Vorbild der Schweizer HIV- und AIDS-Kohorten-Studie, an der sich einer unserer Preisträger, Prof. Amalio Telenti, federführend beteiligte. Die Stiftung Prof. Dr. Max Cloëtta wird sich immer wieder dafür einsetzen, nicht nur hervorragend qualifizierten biomedizinischen Grundlagenforschern eine akademische Karriere zu ermöglichen, sondern auch Medizinern für fünf Jahre die Freiheit zu geben, innovative Forschung zu betreiben und während dieser Zeit nicht von der klinischen Routine erdrückt zu werden. Mit dieser Starthilfe sind sie in darauf folgenden Abschnitten ihrer Karriere in der Lage, international anerkannte klinische Forschung – wenn nicht selber durchzuführen – so doch kompetent motivieren und leiten zu können.

Unsere Preisträger sind dieses Jahr beide Mediziner und auch klinisch tätig. Beide haben sich aber auch in biomedizinischen Grundlagenfächern ausbilden lassen. Der eine hat seine Dissertation in Biochemie gemacht, der andere mit einer Zusatzpromotion in Mikrobiologie abgeschlossen. Mit dieser Doppelausbildung haben sie, jeder für sich, den Grundstein für ihre bahnbrechenden Arbeiten gelegt, die nun zweifelsohne der Klinik zugute gekommen sind.

Professor Amalio Telenti ist Direktor und Médecin chef vom Institut de Microbiologie am CHUV in Lausanne. Er hat als erster den Grund für die Resistenz von Tuberkulose-Erregern gegen drei verschiedene

Antibiotika gefunden, was nun erlaubt, dem individuellen Patienten das richtige Antibiotikum zu verabreichen. Auch im Aids-Gebiet gelangen ihm und seiner Gruppe wichtige Beiträge zum Verständnis der Resistenz gegen antivirale Therapien. Es freut mich, dass Professor Luc Perrin, Direktor des Laboratoire de Virologie in der Division des Maladies Infectieuses am Genfer Universitätsspital, Professor Telentis Beiträge würdigen wird.

Professor Radek C. Skoda ist Direktor des Departementes Forschung des Basler Universitätsspitals und gleichzeitig Vizedekan für Forschung der Medizinischen Fakultät Basel. Sein wichtigster Beitrag ist die erste Abklärung des molekularen Mechanismus, der zu Thrombozythaemie führt. Das ist wohl eine seltene Krankheit. Mit der Entdeckung aber, dass Proteintranslations- und nicht Genexpressionsfehler – insbesondere Fehler der Überproduktion – Ursache einer Krankheit wie der Thrombozythaemie sein können, hat Prof. Skoda ein neues Kapitel über pathogenetische Mechanismen aufgeschlagen. Ich danke Professor Kaspar Winterhalter aus Zürich, einem alten Freund und molekular orientierten Hämatologen der ersten Stunde, dass er die Laudatio für die Beiträge von Prof. Radek C. Skoda halten wird.

Funktionieren und Erfolg von Stiftungen, wie der Stiftung Prof. Dr. Max Cloëtta, beruhen auf drei Säulen. Erstens dem fünfköpfigen medizinischen Ausschuss, der in fünf Sitzungen und Konferenzen im Jahr die beiden Preisträger und die mehrjährigen Forschungsstelleninhaber vorschlägt. Zweitens den drei übrigen Mitgliedern des Stiftungsrates, der alle Entscheidungen, auch jene, die das Stiftungsvermögen betreffen, fällt. Diese beiden Gremien arbeiten ehrenamtlich. Und keineswegs letztlich der dritten Säule, der Geschäftsstelle, welche die Entscheidungen ausführt und beispielsweise auch für den reibungslosen Ablauf dieser Preisverleihung sorgt.

Meinen sieben Kollegen im medizinischen Ausschuss wie auch im übrigen Stiftungsrat, genauso wie Frau Brigitt Küttel und Frau Irène Thomann von der Geschäftsstelle, möchte ich für ihre Treue der Stiftung gegenüber und für ihren erheblichen Einsatz durch das ganze Jahr hindurch sehr herzlich danken.

LE PRIX CLOËTTA 2004
EST DÉCERNÉ À

MONSIEUR LE PROFESSEUR

AMALIO TELENTI

NÉ EN 1959 À OVIEDO (ESPAGNE)
PROFESSEUR DE VIROLOGIE MEDICALE,
CHEF DE L'INSTITUT DE MICROBIOLOGIE
AU CENTRE HOSPITALIER UNIVERSITAIRE VAUDOIS,
POUR SES REMARQUABLES TRAVAUX CONCERNANT LE SIDA.

ZURICH, LE 26 NOVEMBRE 2004

AU NOM DU CONSEIL DE FONDATION:

LE PRÉSIDENT

LE VICE-PRÉSIDENT

UN MEMBRE

Prof. Dr. Amalio Telenti

CURRICULUM VITAE

Name:	Amalio Telenti
Place and date of birth:	Oviedo, Spain; 11 October 1959
Citizenship:	Spain
Marital status:	Married, 2 children

Education and Training:

1982	Graduate in Medicine, Medical School. University of Oviedo, Spain.
1983	Medical Clerkship. Mayo Medical School. Rochester, MN, USA. Certification, Visa Qualifying Examination (ECFMG).
1984–1987	Resident in Internal Medicine, Mayo Clinic. Rochester, MN, USA.
1987	American Board of Internal Medicine (Internal Medicine).
1987–1990	Fellow in Infectious Diseases, Mayo Clinic. Rochester, MN, USA.
1990	American Board of Internal Medicine (Infectious Diseases).
1991–1993	Fellow, Institute for Medical Microbiology, University of Berne, Switzerland.
2000	PhD, Microbiology, University of Zaragoza, Spain.

Appointments

1994–1997	Oberarzt, Institute for Medical Microbiology, University of Berne, Switzerland.
1995	Privatdozent Medical Faculty, University of Bern, Switzerland.
1995	Research Associate, Howard Hughes Medical Research Institute. Dep. Microbiology and Immunology Albert Einstein College of Medicine, Bronx, New York. USA.
1997–2003	Chief, HIV Unit, Médecin adjoint, Div. of Infectious Diseases, Centre Hospitalier Universitaire Vaudois. Médecin associé, Institute of Microbiology, University of Lausanne, Switzerland.
2000	Privat Dozent et Maître d'enseignement et recherche Medical Faculty, University of Lausanne, Switzerland.
2002	Associate Professor of Infectious Diseases Medical Faculty, University of Lausanne, Switzerland.
2003	Professor of Medical Virology University of Lausanne, Switzerland.

Honors

1982	University of Oviedo, Medical Faculty Award.
1989	Mayo Foundation "J. Geraci" Award in Infectious Diseases.
1990	Mayo Foundation "Summerskill" Award for Research in the Department of Internal Medicine.

1993	Swiss Society of Infectiology. “MSD-Chibret Prix de la Recherche Clinique ou Expérimentale en Infectiologie”.
1993	University of Berne. Award of the Medical Faculty “Diagnostic and therapeutics”.
1994	Cleveland Clinic Foundation: International Visiting Lecturer.
1997	European Society of Clinical Microbiology and Infectious Diseases. Young Investigator Award for Research.
2002	Mayo Clinic. Distinguished Speaker – Medical Grand Rounds.
2002	Leenaards Award.
2004	Prize Prof. Dr. Max Cloëtta.

Current Expert Boards

– Member, Scientific Board of the Swiss HIV Cohort Study.

– Member, Clinical Specialized Commission for AIDS, Swiss Federal Office for Public Health.

– Faculty, antiretroviral resistance expert panel – International AIDS Society USA.

– Editor, Antiviral Therapy.

– Member, Comité CSS3, Agence National de la Recherche SIDA-France.

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ADAPTATION, CO-EVOLUTION, AND HUMAN SUSCEPTIBILITY TO HIV-1 INFECTION

Amalio Telenti

«The closer you look at life, the more rapid and intense the rate of evolutionary change.»

Jonathan Weiner, The Beak of the Finch

Darwin believed evolution occurred over very long periods of time and generally moved in a set direction toward fitness, in the same direction the environment was heading. HIV-1, by means of its inherent capacity to mutate, and thus evade and adapt, serves to assess co-evolution with humans over the short period that elapsed since the estimated entry of HIV-1 into the human population during the first half of the last century (Korber, 2000). In this paper, I review the selective pressures exerted by humans on the virus, and potential evolutionary influences exerted by the virus on the human population under the weight of the current pandemic. I will pay special attention to the growing knowledge on the elaborate innate defense mechanisms against retroviruses, and on the potential role of human genetic variants of antiviral genes and of host proteins needed for the viral life cycle in modulating human susceptibility to HIV-1 infection.

Adaptation of HIV-1 to the human population

Humans exert powerful selective pressures on HIV-1. Some are exogenous in nature, such as the pressure exerted by antiretroviral drugs leading to the selection of drug resistance, and others are of endogenous nature, such as the pressure generated by the adaptive immune system, leading to selection of immune escape mutants. These two processes shape the virus in a fashion that may lead to population-specific HIV-1 variants, and that generally result in diminished viral fitness. In population biology, fitness is defined by the relative reproductive capacity of an individual based on its contribution to the next generation. In viro-

logical terms, fitness is best described by the relative replication rates of viruses in competition kinetics (Bleiber, 2001b).

Adaptation to drug pressure. Although the fitness of resistant HIV-1 strains overlaps that of susceptible clinical isolates, resistant strains are overall less infectious and replication competent (Telenti, 1999, Kaufmann, 2000, Bleiber, 2001a, Bleiber, 2001b). Mutations selected by reverse transcriptase (RT) and protease (PR) inhibitors frequently involve changes of enzyme active site residues. Reduction in primer extension activity by mutant RT has been demonstrated in vitro (Back, 1996, Back, 1997). Mutant PR enzymes exhibit diminished catalytic efficiency in the processing of viral Gag and Gag-Pol polyproteins (Schock, 1996, Zhang, 1997, Rayner, 1997, Croteau, 1997, Carrillo, 1998). These modifications in enzyme processivity may translate into the accumulation of immature viral particles. Kinetic analysis of consecutive proteolytic cleavages of the Gag-Pol polyprotein suggests that HIV-1 would cease being viable when the efficiency of a mutant protease is less than 61% of the wild type activity for each step of cleavage (Rasnick, 1997). Mutation in both PR and RT can contribute to diminished viral fitness, and either enzyme may display a profound impairment (Bleiber, 2001a). Resistance to HIV-1 fusion inhibitors is also associated with impaired fitness, while resistance to non-nucleoside reverse transcriptase inhibitors (NNRTI) modifies viral physiology only minimally (Schmit, 1996, Rayner, 1997). This has been attributed to drug binding remote from the RT active site (Esnouf, 1995), and explains the occasional identification of NNRTI resistance mutations as natural polymorphisms (Havlir, 1996).

Given the remarkable plasticity of the HIV-1 genome, selection of compensatory mutations leading to improving enzyme function takes place. This process involves structurally relevant amino acid substitutions in target enzymes (e.g., in the hinge or flap regions of the viral protease) (Schock, 1996, Borman, 1996), changes that improve processing of enzyme substrate (e.g., Gag cleavage sites) (Doyon, 1996, Zhang, 1997, Carrillo, 1998, Bally, 2000), or by compensation at distance (Nijhuis, 1999, Peters, 2001). However, these pathways of compensation may not provide an immediate or full remediation of viral fitness deficits (Mammano, 1998)

as there could be evolutionary and adaptive limits of HIV-1 (Yuste, 1999, Nijhuis, 1999). Diminished fitness underlies the lower pathogenicity of multidrug-resistant strains (Kaufmann, 1998). Fitness cost can also be translated into transmissibility cost. Resistant strains, and in particularly multidrug-resistant viruses are less transmissible in a population (Yerly, 2004), a phenomenon that can be ascribed to adaptive attenuation.

Adaptation to immune pressure. Immune selective pressure forces the emergence of viruses with escape mutations that result in infected cells not being recognized by cytotoxic T lymphocytes (CTL) (Lieberman, 2002). Viruses adapt to the host they infect through a process of HLA-associated selection (Moore, 2002). This carries a fitness cost, as proven by reversion after viral transmission to a different host, when the CTL pressure exerted on the virus shifts (Leslie, 2004). This process, when brought to the scale of the population, implies that after many cycles of viral replication and passage in human hosts, the viral population evolves to a consensus virus with mutations in genes that encode dominant immunogenic peptides (Scherer, 2004). MHC-restricted immune responses may shape the viral genetic diversity over time, providing a selective pressure that is a function of the frequency of various alleles as the virus is passed from one host to the next. In this scenario, it may be predicted that the most common viral population (ie the 'population consensus sequence') is likely to represent the genotype best adapted to the most frequently encountered disease-modifying MHC alleles (or haplotypes). Trachtenberg et al. (Trachtenberg, 2003) proposed the occurrence of frequency-dependent selection according to HLA supertypes, an attractive hypothesis (Telenti, 2003) that has recently gained support with the identification of a higher capacity to elicit CTL responses by rare HLA supertypes (Scherer, 2004). Eventually, pathogen diversity and discrete strain structure may be determined by different host networks (Buckee, 2004).

Adaptation of the human population to HIV-1

While the response of the virus to selective pressures imposed by the host can be readily observed, it is much more difficult to define the evo-

lutionary pressures of the virus on the host because of the recent nature of the epidemic. However, this topic can be revisited if what is described is the adaptation of humans to retroviruses. To approach this topic, I will focus on the determinants of human susceptibility to HIV, and the genetic basis of inter-individual differences in susceptibility to disease progression.

Interindividual levels of permissiveness. The genetic make-up of an individual plays a role in his or her susceptibility to HIV-1 infection and progression of disease. Some of the observed variation has been attributed to immunogenetic diversity (MHC homozygocity, specific HLA types), polymorphism in chemokines, chemokine receptors and in cy–tokine genes *(CCR5, CCR2, CX3CR1, SDF1, MIP1a, RANTES, IL-10, IL-4)* (Carrington, 2001, Telenti, 2002, O'Brien, 2004). Isolated primary human cells from different individuals also vary in their permissiveness – the ability of purified cells to be infected and to sustain replication of HIV-1 (Williams, 1991, Spira, 1995, Eisert, 2001, Ciuffi, 2004). Infection of macrophages derived from pairs of identical twins displays a high concordance in the kinetics of HIV-1 replication (Chang, 1996, Naif, 1999), underscoring the role of genetic factors in determining susceptibility to infection.

The HIV-1 life cycle is characterized by numerous interactions with host cellular proteins (Greene, 2002, Peterlin, 2003) *(Figure 1)*. Restriction at entry plays a key role in determining infection kinetics (Reeves, 2002, Butticaz, 2003). However, host proteins involved in later steps of the viral life cycle also contribute to interindividual susceptibility to HIV-1 infection (Ciuffi, 2004). Genetic polymorphism in host genes participating in the viral life cycle could result in differences in the levels of expression or in functional differences of protein variants that may lead to differences in permissiveness to HIV-1 infection. The cell would thus constitute an "environment of evolution", whereby differences among individuals or inter-species, lead to viral variants adapted to different host partner proteins. Eventually, escape mutants with potential fitness deficits or increase pathogenicity may contribute to shape the epidemic. One of the main focuses of the research in my laboratory has been to understand interindividual differences in

susceptibility to HIV-1 by developing *in vitro* systems for the standardized assessment of the contribution of genetic variants of selected host genes, and that would allow the genomic mapping of determinants of susceptibility to HIV-1.

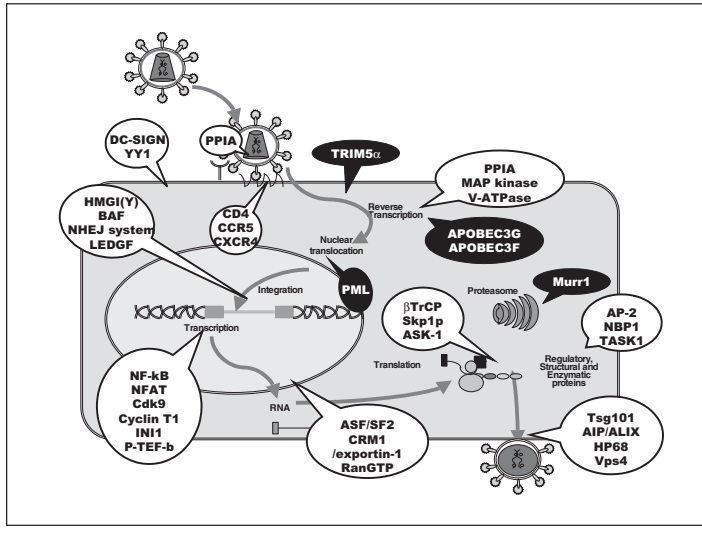

Figure 1:
The HIV-1 life cycle. Indicated are host proteins that participate at specific steps of the replication cycle (white), and proteins acting as antiretroviral host factors (black).

The first goal was approached by defining the steps in the viral life cycle that exhibit the most variation between individuals (Ciuffi, 2004). We used replicating HIV-1 and single cycle lentiviral vectors in a population approach to identify polymorphic steps during viral replication. We found that stimulated CD4 T cells (the main cellular target of HIV-1) exhibited up to 5 log unit differences in virus production. We were able to attribute up to 42% of the total variance in virus production to entry factors, and 48% to post-entry steps. Efficacy at key intracellular steps of the replicative cycle (reverse transcription, integration, trans-

cription and splicing, translation, and budding and release) varied among individuals. However, differences in transcription efficiency alone explained up to 80% of the total variance in viral production that was attributable to post-entry factors. The observation that transcription polymorphism has a weight comparable to that of entry variation should trigger a detailed analysis of genes participating in the activation, transcriptional status, and transcriptional machinery of the cell. This observation is set in the context of current awareness of the role of variation in human gene expression underlying complex traits (Cox, 2004, Morley, 2004).

The development of this well-characterized in-vitro system allowed us to use it as screening tool for HIV-1 restriction alleles (Bleiber, 2004). Susceptibility to HVI-1 is a complex trait compounded by unknown environmental and genetic factors pertaining to both the human host and the virus. To circumvent some of these difficulties we proposed a two-step strategy, in which distinct alleles in potential candidate genes are first assessed *in vitro* for their impact on viral replication, and subsequently validated *in vivo* for their role in restricting HIV-1 infection. As a benchmark test we first tested 12 previously reported HIV-1 restriction alleles as well as single nucleotide polymorphisms in 10 additional candidate genes. This led to the identification of 3 new alleles associated with differences in progression of HIV-1 disease in the Swiss HIV Cohort Study. We estimate that the genetic effects of these markers might be responsible for lengthening or shortening the latency period to AIDS by up to 4.6 years. It is to facilitate this type of studies that we proposed the creation of the Genetics Project of the Swiss HIV Cohort Study (Telenti, 2004, Furrer, 2004).

To complement the approach using CD4 T cells from blood donors, we are now using immortalized B lymphocytes from the Centre d'Etude du Polymorphisme Humain (CEPH) Utah pedigrees (Dausset, 1990). Here, quantitative traits can be mapped to chromosomal locations by genome scans (Schork, 2002, Jen, 2003, Cheung, 2003, Pastinen, 2004, Morley, 2004, Watters, 2004). While susceptibility to HIV-1 cannot be approached *in vivo* by linkage analyses, but only through association studies, the CEPH resource allows conventional linkage analysis for quan-

titative traits. These cells also allow the re-assessment of haplotypes that are possibly associated with differences in gene expression, by the measurement of allele differential expression (Yan, 2002, Pastinen, 2004).

Innate cellular defense. While certain host proteins are necessary for infection and for sustaining viral replication, others represent antiviral factors *(Figure 1)*. Some cellular antiviral factors can be selectively suppressed by viral proteins, as shown by the interaction between human APOBEC3G, a cytidine deaminase, and the HIV-1 Vif protein (Sheehy, 2002, Mariani, 2003, Harris, 2003, Mangeat, 2003, Lecossier, 2003). An early interplay between incoming retroviral preintegration complexes and the nuclear proteins INI1 (integrase interactor 1) and PML (promyelocytic leukemia protein) creates an antiviral state that interferes with the immediate-early steps of HIV-1 infection (Turelli, 2001). ZAP, a zinc finger protein, inhibits production of retroviral RNA (Gao, 2002). Other antiretroviral factors target the capsid protein, imposing a post-entry block (Hatziioannou, 2003, Stremlau, 2004). Murr1, a gene product known previously for its involvement in copper regulation, acts as a genetic restriction factor that inhibits HIV-1 growth in unstimulated CD4+ T cells through its effects on the proteasome (Ganesh, 2003).This growing list of specific antiretroviral factors adds to the current knowledge on antiviral innate defense mechanisms that implicate interferon responses mediated by double stranded RNA-dependent protein kinase, the MX proteins, and RNase L-mediated degradation of viral RNAs (Goff, 2003).

Two antiviral proteins, APOBEC3G and TRIM5α, underscore the concept that the biological barrier preventing the entry of simian immunodeficiency viruses into the human population as zoonotic infections is potentially quite fragile. A single APOBEC3G residue, aspartic 128 in human, lysine in African green monkeys controls the ability of the HIV-1 Vif protein to bind and inactivate this host defense factor (Schrofelbauer, 2004, Mangeat, 2004). HIV-1 efficiently enters the cells of Old World monkeys but encounters a block before reverse transcription by modulating the uncoating of a retroviral capsid (Stremlau, 2004). The cytoplasmic body component TRIM5α restricts HIV-1 infection in Old World monkeys while the simian immuno-

deficiency virus, which naturally infects Old World monkeys, is less susceptible to the TRIM5α-mediated block. Host cell barriers may largely explain the current distribution of these viruses among human and non-human primate species (Lee, 2004).

We have investigated variation in these antiviral proteins (Bleiber, 2004, An, 2004) and identified a significant polymorphism, including the occurrence of nonsynonymous amino acid substitutions in defined protein motifs. This would indicate that these innate defense elements are under significant selective pressure (Sawyer, 2004, Zhang, 2004). A histidine 186 to arginine change in APOBEC3G, frequently identified in individuals of African ancestry, may be associated with more rapid progression of HIV-1 disease (An, 2004). TRIM5α variants are, in the *in vitro* assay used in our laboratory, associated with differences in the degree of permissiveness of CD4 T cells to HIV-1 infection. Exploring differences in expression or function of these antiviral proteins may shed light on the basis of inter-individual or inter-species differences in susceptibility to retroviruses, and possibly other pathogens (Turelli, 2004).

Perspectives

The various processes described above lead us to the last issue, whether we are witnessing HIV-1-host co-evolution. The best evidence for the influence of human pathogens on natural selection is exemplified by Malaria (Winkler, 2004). This disease became endemic 6000–10 000 years ago, coincident with the rise of agriculture (Tishkoff, 2001, Joy, 2003). The selective pressure over 300–500 generations has resulted in adaptive shifts in the allele frequency of several genes with a role in malaria resistance: the X-linked glucose-6-phosphate dehydrogenase *(G6PD)*, the Duffy antigen receptor for chemokines *(DARC)*, and the α and β-globin genes (Fortin, 2002). Another suggestive example of population shaping constitutes the *FUT2* – fucosyltransferase 2 and resistance to Norovirus infection. *FUT2* encodes α1,2 fucosyltransferase that produces H type 1 carbohydrate found in the surface of the intestinal epithelium, required for Norovirus binding (Lindesmith,

2003). As regards to HIV-1, the only well characterized human population escape mutants are individuals who are homozygous for CCR5 Δ32. The resulting truncated chemokine receptor provides resistance to HIV-1 infection and is present in approximately 2% of Caucasian individuals. However, *CCR5 Δ32* was selected approximately 700 years ago, not by HIV, but possibly by another pathogen (Stephens, 1998, Elvin, 2004, Mecsas, 2004). If no effective control of the HIV-1 pandemic is achieved, the continuing pressure may give rise to a formal selection of a HIV-resistant human population.

For years researchers have been studying the determinants of acquired immunity to HIV-1; and with the same intensity and funding we should now define the cellular determinants of susceptibility to HIV-1. Our work will continue to probe the genetic basis of interindividual differences in retroviral restriction. This information is to be placed in the context of the primate phylogeny, through detailed comparative genomics.

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I wish to express my appreciation to the Cloëtta Foundation for having distinguished my work. I accept this honor as a recognition of the rewarding but difficult task of being both a clinician and a physician engaged in laboratory research. Rewarding because, over the years, I have been surprised by many observations in the laboratory and by how they have shaped my understanding of clinical medicine. I also thank The Swiss National Science Foundation for 10 years of continuous support.

I dedicate this award to the memory of my father and grandfather, physicians caring for patients with infectious diseases. My very special thanks to my wife Anen, to my mother, and to Nicolás and Adriana.

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DER CLOËTTA-PREIS 2004
WIRD VERLIEHEN

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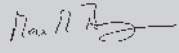
ZÜRICH, 26. NOVEMBER 2004

FÜR DEN STIFTUNGSRAT:

DER PRÄSIDENT



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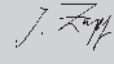

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2000	International Society for Experimental Hematology.
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2001	External reviewer for INSERM, Paris, France.

2003	External reviewer for The Wellcome Trust, UK.
2004	Co-chair of the Scientific and Educational Committee for the 9th Meeting of the European Society of Haematology (EHA-9).
2004	Member of the Scientific Committee of the European School of Haematology (ESH).

KEY PUBLICATIONS

1. Skoda, R.C., Seldin, D.C., Chiang, M., Peichel, C.L., Vogt, T.F., and Leder, P. (1993) Murine c-*mpl*, a member of the hematopoietic growth factor receptor superfamily that transduces a proliferative signal. *EMBO J.*, 12:2645–2653.

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3. Stoffel, R., Wiestner, A., and Skoda, R.C. (1996) Thrombopoietin in thrombocytopenic mice: evidence against regulation at the mRNA level and for a direct regulatory role of platelets. *Blood*, 87:567–573.

4. Ghilardi, N., Ziegler, S., Wiestner, A., Stoffel, R., Heim, M.H., and Skoda, R.C. (1996) Defective STAT signaling by the leptin receptor in *diabetic* mice. *Proc. Natl. Acad. Sci. USA*, 93:6231–6235.

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7. Kralovics, R., Buser, A.S., Teo, S.S., Coers, J., Tichelli, A. van der Maas, A.P.C., Skoda, R.C. (2003) Comparison of molecular markers in a cohort of patients with myeloproliferative disease. *Blood*, 102:1869–1871.

8. Schomber, T., Kalberer, C., Wodnar-Filipowicz, A., Skoda, R.C. (2004) Gene silencing by lentivirus-mediated delivery of siRNA in human $CD34^+$ cells *Blood*, 103:4511–4513.

9. Coers, J., Ranft, C., and Skoda, R.C. (2004) A truncated isoform of c-mpl with an essential C-terminal peptide targets the full-length receptor for degradation. *J. Biol. Chem*. 279:36397–36404.

THE CONTROL OF NORMAL AND ABERRANT MEGAKARYOPOIESIS BY THROMBOPOIETIN AND ITS RECEPTRO, c-MPL

Radek C. Skoda

Introduction

Mature blood cells are short-lived and must be constantly replaced. A small number of multipotent hematopoietic stem cells (HSC) are the source of this regenerative process. Hematopoiesis is the best studied stem cell system and constitutes a model for other organs. HSC can either remain multipotent or differentiate into mature cells with very diverse functions and morphologies, as exemplified by lymphocytes, granulocytes, erythrocytes or megakaryocytes. Extracellular signals, such as cytokines and growth factors and their cell surface receptors, act in concert with cell intrinsic factors, e.g. components of the signal transduction machinery and transcription factors, to maintain a balance between production and destruction of blood cells. Given the complexity of the known regulatory pathways and the needs for adaptation to changing environmental factors, we should consider it surprising that blood cell disorders are not more common. Here I review the physiological role of a humoral factor called thrombopoietin and its receptor in the formation of megakaryocytes and platelets and I summarize our current understanding of disorders associated with an overproduction of platelets.

Thrombopoietin and its receptor, c-Mpl are the primary regulators of platelet production

Thrombopoietin (TPO) was first described as an biological activity in serum that stimulated the production of platelets in vivo (Kelemen et al., 1958). For more than 30 years this activity eluded all attempts of biochemical purification. TPO cDNA was finally cloned in 1994 by virtue of its high affinity receptor, which served as a bait for immuno-

affinity purification and expression cloning (Bartley et al., 1994; de Sauvage et al., 1994; Kaushansky et al., 1994). The TPO receptor was discovered as the cellular homologue of a retroviral oncogene. Experiments with Friend murine leukemia virus led to the isolation of a mutant virus called myeloproliferative leukemia virus (MPLV) (Wendling et al., 1986). MPLV caused a broad spectrum of myeloid malignancies in mice including erythroid, granulocytic, monocytic, megakaryocytic and mast cell leukemias (Wendling et al., 1989). This recombinant retrovirus carried a novel oncogene, v-mpl, that displayed sequence homology to members of the cytokine receptor superfamily. A rearrangement led to a fusion between sequences coding for the extracellular domain of the retroviral envelope protein and a truncated cytokine receptor, consisting of 40 amino acids of the extracellular domain, the transmembrane domain, and the entire cytoplasmic domain (Souyri et al., 1990). This suggested that signaling through the cytoplasmic domain of v-mpl was responsible for the proliferation and transformation of hematopoietic progenitors. Since I was interested in megakaryocyte formation and transformation, I began to study the properties of c-mpl, the cellular homologue of v-mpl, which at this time was an orphan receptor molecule. To demonstrate that c-mpl was capable of signaling for proliferation, I generated a chimeric receptor consisting of the extracellular domain of the interleukin-4 (IL-4) receptor and the transmembrane and cytoplasmic domain of c-mpl. When stimulated by IL-4, the cytoplasmic domain of the c-mpl protein was capable of providing a proliferative signal in factor-dependent cell lines (Skoda et al., 1993). The full-length c-mpl protein and a soluble extracellular domain was used by several laboratories to clone the mpl-ligand, which turned out to be the long sought for TPO (Bartley et al., 1994; de Sauvage et al., 1994; Kaushansky et al., 1994). TPO causes thrombocytosis in vivo (Kaushansky et al., 1994) and promotes proliferation and maturation of megakaryocyte precursors in vitro (Debili et al., 1995; Kaushansky et al., 1995). TPO is mainly synthesized in the liver and the control of TPO production provides an interesting paradigm for autoregulation (Kuter and Rosenberg, 1995). In support of this model (Figure 1), we found that TPO mRNA was not regulated during thrombocytopenia. Furthermore, isolated mouse platelets absorbed high amounts of bioactive TPO out of TPO-conditioned medium in a

dose-dependent fashion (Stoffel et al., 1996). Thus, serum TPO protein is being regulated directly through adsorption by platelets *(Figure 1)*. Consistent with this model, platelets deficient for c-mpl were unable to bind and absorb TPO (de Sauvage et al., 1996; Gurney et al., 1994).

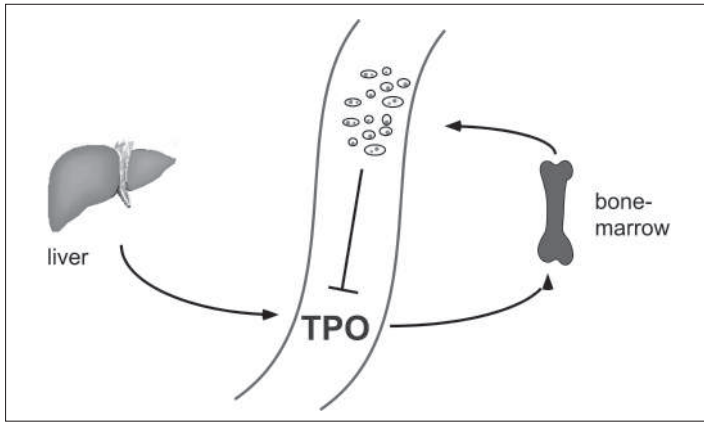

Figure 1:
Autoregulation of TPO serum levels. The liver produces constant amounts of TPO, which is transported to the bone marrow through the blood vessels. TPO leads to an increase of platelet production and results in a higher platelet count. Platelets express the TPO-receptor. They are able to bind and degrade circulating TPO protein and thereby exert a negative feedback.

One very interesting question is how specificity of the in vivo effects of TPO, i.e. the selective increase of platelets in the peripheral blood, can be explained. Since c-mpl is expressed on early hematopoietic progenitors and stem cells, one possibility is that signals generated by TPO and its receptor act by instructing the progenitors to commit to a specific hematopoietic lineage (instructive model). Alternatively, lineage specificity could be also achieved by restricting the expression of the receptor to cells of the appropriate lineage. According to this theory, the signals generated by the ligand-receptor interaction primarily permit the survival and proliferation of pre-determined progenitors through a

non-specific proliferative signal (permissive model). To address this question in vivo, we used homologous recombination to replace the c-mpl gene with a chimeric construct encoding the extracellular domain of mpl and the cytoplasmic domain of the granulocyte colony-stimulating factor receptor (G-CSFR) *(Figure 2)*. This chimeric receptor binds TPO, but signals through the G-CSFR intracellular domain (Stoffel et al., 1999). We found that, despite the absence of a functional mpl signaling domain, homozygous knock-in mice had a normal platelet count, indicating that in vivo the cytoplasmic domain of G-CSFR can functionally replace mpl signaling to support normal megakaryopoiesis and platelet formation *(Figure 2)*. This finding favors the permissive model, according to which cytokine receptors provide a non-specific survival or proliferation signal and argues against an instructive role of c-mpl or G-CSFR in hematopoietic cell fate decisions. Similar conclusions were reached in a knock-in model with a G-CSFR/EPOR chimera (Semerad et al., 1999).

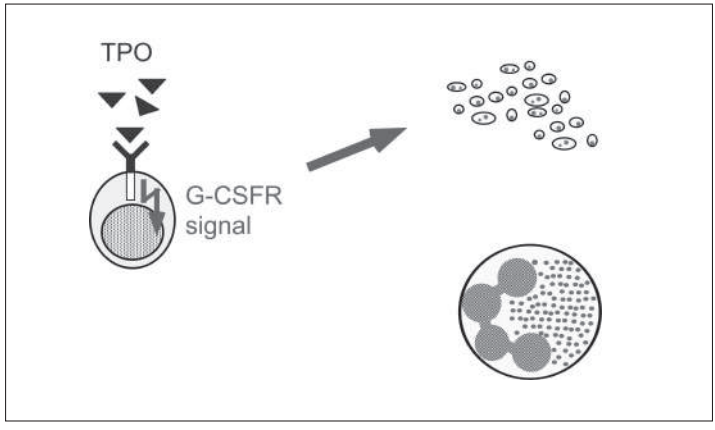

Figure 2:
Summary of the conclusions reached from the analysis of homozygous mpl/G-CSFR knock-in mice. The chimeric mpl/G-CSFR binds TPO but signals through the G-CSFR cytoplasmic domain. The resulting signal does not re-program the progenitor cells to become granulocytes, instead this signal is capable of promoting megakaryopoiesis and platelet production in vivo.

Since mice deficient for mpl are viable, they can be used as a genetic background to introduce mutant receptors and assess their capability to complement the platelet deficiency in vivo. We have introduced transgenes encoding c-mpl mutants into c-mpl-deficient mice and found that these mutants change platelet levels, but not platelet function or the balance between cells of different hematopoietic lineages (Coers et al., in preparation). These results are again consistent with the predictions of the permissive model.

Recently, we have studied the function of a truncated mpl receptor isoform (mpl-tr), which results from alternative splicing (Skoda et al., 1993). The mpl-tr variant is the only alternate mpl isoform conserved between mouse and humans, suggesting a relevant function in regulating mpl signaling. Despite the presence of a signal peptide and the lack of a transmembrane domain, mpl-tr is retained intracellularly. Our results provide evidence that mpl-tr exerts a dominant-negative effect on thrombopoietin-dependent cell proliferation and survival (Coers et al., 2004). We demonstrated that this inhibitory effect is due to down-regulation of the full-length mpl protein. The C terminus of mpl-tr, consisting of 30 amino acids of unique sequence, is essential for the suppression of TPO-dependent proliferation and mpl protein down-regulation. Cathepsin inhibitor-1 (CATI-1), an inhibitor of cathepsin-like cysteine proteases, counteracts the effect of mpl-tr on mpl protein expression, suggesting that mpl-tr targets mpl for lysosomal degradation. Together, these data suggest a new paradigm for the regulation of cytokine receptor expression and function through a proteolytic process directed by a truncated isoform of the same receptor. This example highlights the many levels of fine-tuning nature invented to obtain a robust regulatory system for maintaining stable numbers of circulating blood cells.

Aberrant megakaryopoiesis in hereditary forms of myeloproliferative disorders.

Myeloproliferative disorders (MPD) are a heterogeneous group of diseases characterized by increased hematopoiesis leading to elevated

numbers of non-lymphoid cells and/or platelets in the peripheral blood. In addition to thrombotic and hemorrhagic complications, leukemic transformation can occur. The classical definition of MPD included four disease entities, which were thought to be related (Damashek, 1951): chronic myelogenous leukemia (CML), polycythemia vera (PV), essential thrombocythemia (ET) and primary myelofibrosis (IMF). The Philadelphia chromosome and the bcr/abl fusion transcripts established CML as a separate entity, allowed developing diagnostic PCR tests and made it possible to derive a specific tyrosine kinase inhibitor (Imatinib/ Glivec), which is used successfully in the treatment of CML patients. The cause of the three remaining forms of MPD remains unknown, but progress has been made in defining the functional and molecular characteristics of MPD. The investigation of inherited disorders resembling MPD complements these studies and provides important clues to the hierarchy of events in the pathogenesis of MPD.

The finding that TPO and its receptor exert strong lineage selective effects on platelet production suggested that mutations in TPO or c-mpl could play a role in disorders with increased or decreased megakaryopoiesis. Inherited disorders are particularly suited to test such a hypothesis, because the involvement of a candidate gene locus can be confirmed or excluded by genetic linkage analysis. In a family with autosomal dominant thrombocytosis resembling ET, we were able to locate the disease-causing gene to the TPO locus on chromosome 3q and to find a mutation in the TPO gene (Wiestner et al., 1998). The mechanism of how this mutation causes elevated TPO serum levels was at first quite difficult to understand. Affected family members carry a point mutation in the +1 position of the splice donor of intron 3. This mutation causes exon-skipping and results in loss of exon 3, including the nucleotides encoding the first 4 amino acids of the TPO protein. This mutation appeared to cause a loss of function of the TPO allele, thus, the opposite of what we had expected. However, more detailed analysis revealed that a novel N-terminus is created by fusion with an upstream open reading frame and we could show that the extended N-terminus behaves as a functional signal peptide. Importantly, the mutation results in TPO overproduction due to removal of sequences contained within exon 3 that normally inhibit the translation of TPO

mRNA into protein. A simplified description of the mechanism, which leads to TPO overproduction in family members that carry the mutation is shown in *Figure 3*.

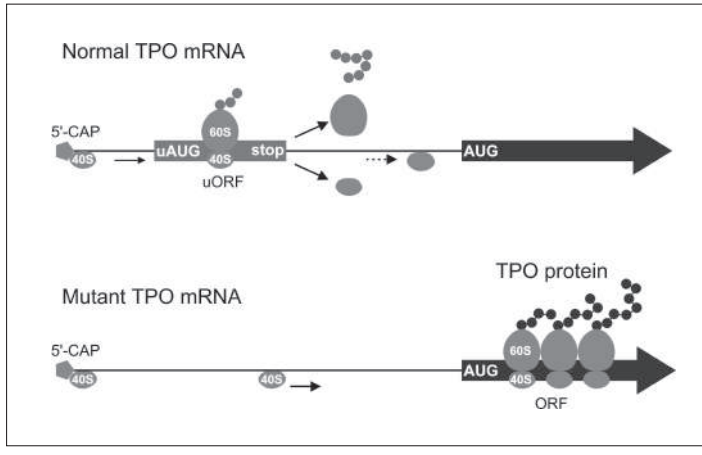

Figure 3:
Mechanism of increased TPO protein production in family members carrying a TPO mutation. Physiologically, the translation of TPO mRNA is strongly inhibited by the presence of an upstream translational start codon (uAUG) located in the 5'-untranslated region (5'-UTR) of the TPO mRNA (Ghilardi et al., 1998). The ribosome (40S and 60S subunits) initiates synthesis of a short peptide and dissociated from the mRNA after encountering a stop codon. Therefore, under normal circumstances, production of TPO protein is very inefficient. In affected family members, exon-skipping removes the uAUG and allows efficient translation (Wiestner et al., 1998). Thus, more TPO protein is synthesized from the same amount of mRNA, leading to increase TPO serum levels. Directed mutagenesis of the uAUG in the TPO mRNA restored full translational efficiency, demonstrating that translational inhibition of TPO biosynthesis is entirely mediated by uORF (Ghilardi et al., 1998).

To date, 3 additional mutations in the TPO gene have been described (Ghilardi et al., 1999; Jorgensen et al., 1998; Kondo et al., 1998). In all cases reported, the inheritance is autosomal dominant and the phenotype is already manifest in childhood or at birth. The clinical findings in these families consist of a high platelet count, markedly elevated

serum TPO levels, but normal red blood cell and normal leukocyte counts. The penetrance is usually 100%, the clinical course is mild with occasional thrombotic or bleeding complications, but without leukemic transformation. In all cases studied, overproduction of TPO is caused by a mechanism of increased translational efficiency for the mutant TPO mRNA (Ghilardi and Skoda, 1999; Ghilardi et al., 1999; Wiestner et al., 1998). These mutations constitute paradigms of translational pathophysiology and are examples of a novel molecular mechanism of human diseseas (Cazzola and Skoda, 2000). Mutations in TPO have not been found in patients with sporadic MPD (Harrison et al., 1998; Taksin et al., 1999). Therefore, thrombocythemia due to gain of function mutations in the TPO gene constitutes a phenocopy of sporadic ET.

Whereas mutations in TPO account for only approx. 20% of familial thrombocytosis (Kunishima et al., 1998; Wiestner et al., 2000), the disease-causing gene still remains unknown in all other cases. Thus, mutations in one or several additional genetic loci can also cause thrombocytosis. Genetic analysis in these families will allow us to identify new components that regulate megakaryopoiesis. The phenotype in some of the families resembles sporadic forms of MPD. We found clonal hematopoiesis in the affected individuals, indicating that a somatic mutation acts in synergy with a predisposition inherited through the germline. The search for the mutated gene in families, which display a phenotype very closely resembling sporadic MPD, appears particularly worthwhile and we expect that the mutated gene will also be of relevance for patients with sporadic MPD. In one large pedigree with MPD-like phenotype, we have located the gene to chromosome 9q and are now searching for the mutation in genes that lie within the co-segregating interval (Kralovics et al, in preparation). Our studies suggest that at least two hits are necessary for phenotypic manifestation of MPD: a pre-disposing mutation in a gene on chromosome 9q and a somatic mutation in a second locus *(Figure 4)*.

Studies on sporadic MPD revealed a number of new molecular markers, such as decreased expression of c-MPL protein in platelets (Moliterno et al., 1998) and PRV-1 mRNA in granulocytes (Temerinac et al., 2000). These alterations in the expression pattern could be valuable as

diagnostic tools or might hold keys to the understanding of the pathogenesis. We compared several of these markers simultaneously in a cohort of patients with MPD and found that the correlation between the markers is lower than reported (Kralovics et al., 2003). In particular, the decreased expression of c-MPL protein was present in less than half of patients with PV or ET and was also found in members of a family with a mutation in the TPO gene. This indicates that decreased c-MPL protein is not involved in the pathogenesis, but rather represents a consequence of high platelet count. Comparing patients with sporadic MPD with family members carrying a known molecular alteration proves to be very helpful for distinguishing primary alterations from secondary events.

Future directions

Genome wide expression studies comparing mature blood cells or progenitors from patients with MPD and healthy controls will undoubtedly improve our picture of the changes that occur during the evolution of MPD. We have performed a high-density microarray expression analysis on RNA from MPD patients, healthy controls and familial MPD-like syndromes and are currently analyzing the data. Methods for screening for subtle chromosomal alterations, such as genome wide microsatellite analysis and chip based comparative genomic hybridization, will complement these studies. One result on which we are following up is the finding of loss of heterozygosity on chromosome 9p (9pLOH), which occurs in 30% of patients with PV and constitutes the most frequent chromosomal aberration in MPD (Kralovics et al., 2003; Kralovics et al., 2002). This somatic mutation could act synergistically with a putative mutation of a gene in chr. 9q. Finally, an interesting aspect of MPD is the suppression by the MPD clone of normal hematopoiesis from stem cells not carrying the MPD mutations. We hypothesize that inhibitory signals, such as the transforming growth factor beta (TGFβ) family of signaling molecules, could be mediating this effect *(Figure 4)*. We will test this hypothesis by inactivating the TGFβ receptors on hematopoietic cells using mouse knockout models or by applying siRNA in human hematopoietic progenitors (Schomber et al.,

2004). By the same approach we will assess the functional consequences of inactivating candidate genes identified in our studies of 9pLOH and familial form of MPD-like disorders. The ultimate goal is to identify the primary molecular defect(s) in MPD and secondary mutations that are associated with disease progression. This information will be used to improve clinical management of disease in these patients and will open possibilities for finding new effective treatments.

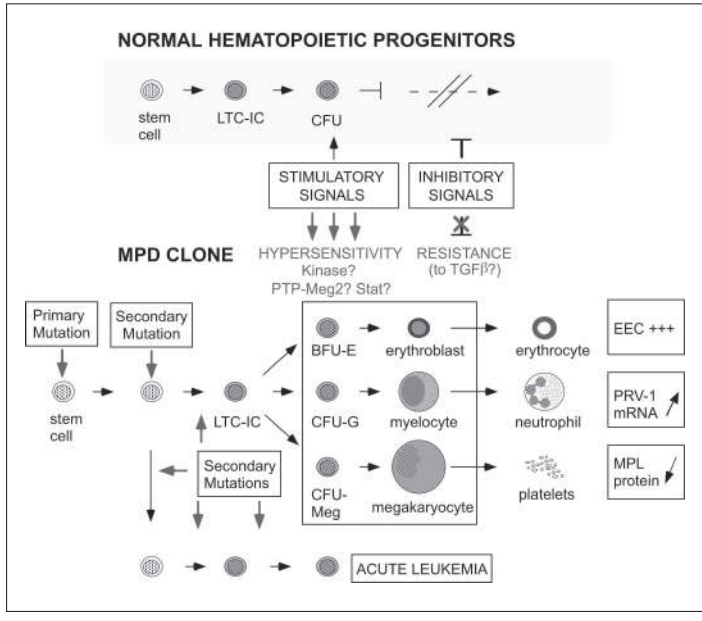

Figure 4:
Hypothetical model for the pathogenesis of myeloproliferative disorders. At least two mutations are needed for phenotypic manifestation of MPD. These mutations convey to cells of the MPD clone hypersensitivity to incoming stimulatory signals and resistance to inhibitory signals. The latter may explain why only blood cells descending from the MPD clone are found in peripheral blood (clonal dominance).

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