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Protection against melanoma by vaccination with Bacille Calmette-Guérin (BCG) and/or vaccinia: an epidemiology-based hypothesis on the nature of a melanoma risk factor and its immunological control

Bernd Krone [a,*], Klaus F. Kölmel [b], Beate M. Henz [c], John M. Grange [d]

[a] *Department of Virology, University of Göttingen, Kreuzbergring 57, D-37075 Göttingen, Germany*
[b] *Dermatological Clinic, University of Göttingen, Germany*
[c] *Department of Dermatology and Allergy, Humboldt University, Charité, Berlin, Germany*
[d] *Centre for Infectious Diseases and International Health, University College London, UK*

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Abstract

A multicentre case-control study conducted by the FEBrile Infections and Melanoma (FEBIM) group has demonstrated a reduced risk of melanoma associated with Bacille Calmette-Guérin (BCG) and/or vaccinia vaccination in early childhood and/or with infectious diseases later in life. This has led to the recognition of a new risk indicator of melanoma; namely 'not being vaccinated with either with BCG or vaccinia'. On the basis of these findings, we propose a hypothesis of immune surveillance for melanoma induced or enhanced by prior contacts with pathogens unexpectedly cross-reactive to a cellular 'marker of melanoma risk'. The reduced risk of melanoma due to BCG and vaccinia, as well as certain common causes of infectious disease, is shown to be associated with antigenic determinants exhibiting sequence homologies with the HERV-K-MEL-antigen. The latter is a product of a pseudo-gene that is closely associated with the env-gene of the endogenous human retrovirus K (HERV-K). A suppressive immune reaction appears to inhibit the expression of endogenous retroviral genes, such as the HERV-K env-gene, that could otherwise result in malignant transformation years or even decades later. The HERV-K env-protein has homologous amino acid sequences with the human nuclear factor Oxygen Responsive Element Binding Protein (OREBP) that controls the expression of glutathione peroxidase. The formation of this and other redox-enzymes, needed to maintain appropriate levels of the normal intracellular redox potential, seems to be suppressed by the OREBP-homologous protein. The present hypothesis is in accordance with the concept that immune dysregulation due to adverse environmental impacts is a risk factor not only for some autoimmune disorders, as previously described, but also for certain malignancies such as melanoma.

Keywords: Melanoma; Tumour; Immune surveillance; BCG; Vaccinia; Vaccination; Selenium

1. Introduction

During the 19th century, independent observations in Germany, England and the United States of America (USA) showed that severe infections such as erysipelas can significantly modify the course of cancer, leading in some cases to regression or even complete resolution of the disease [1]. Against this background, certain bacterial components were used, apparently with some success, in the treatment of cancer during the late 19th and early 20th century. These included treatment of sarcoma with the so-called Coley toxins which are mixtures of

* Corresponding author. Tel.: +49 551 39 5758; fax: +49 551 39 4471.
E-mail address: bkrone@gwdg.de (B. Krone).

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killed *Streptococcus pyogenes* and *Serratia marcescens* [2]. In addition, many attempts have been made to use Bacille Calmette-Guérin (BCG) for the same purpose, and although intravesical BCG for treatment of superficial bladder cancer is of proven benefit [3], results with other cancers have been variable and questionable [4]. It subsequently became apparent from several studies that certain infections and vaccination strategies not only affect the course of established cancers, but also reduce the risk for future development of certain malignancies [5–7]. Interestingly, several hygiene-related factors that are associated with a reduced risk of leukaemia have also been shown to be associated with a reduced risk of atopic and allergic disorders [8].

Epidemiological studies covering several European countries and Israel revealed that vaccinations with BCG and/or vaccinia, and also the occurrence of some uncommon, but severe, infectious diseases, were associated with a significantly reduced risk of subsequently developing melanoma [9–11]. The findings of these epidemiological studies are briefly summarised in Table 1. Moreover, in melanoma patients, the risk of dying was halved over a study period of at least five years in those with a history of BCG and/or vaccinia vaccination [12]. To formulate a testable immunological hypothesis, the following specific questions are raised, analysed and discussed:

1. Is the target of a postulated immune surveillance mechanism an endogenous melanoma risk factor or some kind of malignancy-associated antigen?
2. Is an immune surveillance mechanism for melanoma induced or enhanced by prior contact with pathogens with T cell epitopes unexpectedly cross-reactive with a cellular 'melanoma risk marker'?
3. Is a specific candidate target, sharing sequence homologies with certain microbial antigens, able to elicit an immune surveillance mechanism when presented to the immune system?
4. Are human endogenous retroviruses the basis of the endogenous melanoma risk factor?
5. Does the target-specific immune surveillance suppress endogenous retroviral activity?
6. What directs immune surveillance towards suppressive signalling?
7. How is a suppressive immune response achieved and maintained throughout life?
8. Does the 'melanoma risk protein' disturb the cellular redox regulation, thereby inhibiting apoptosis?

In addition, we pose the question – are there realistic vaccination strategies for the prevention of melanoma? In this context, we identify and discuss possible alternatives to BCG and vaccinia vaccination that could in the future be used to protect the population against melanoma (and possibly other cancers). Some closing remarks are made on the possible underlying evolutionary processes and on the relevance of the hypothesis to the overall maturation and function of the immune system.

2. Is the target of a postulated immune surveillance mechanism an endogenous melanoma risk factor or some kind of malignancy-associated antigen?

The data of the FEBIM study reveal a newly recognised risk factor for melanoma, namely 'not being vaccinated with either BCG or vaccinia' (abbreviated hereafter to 'not vaccinated'). Certain co-variables of 'not vaccinated' were also investigated in parallel in this study. In Table 2, we give some of the previously reported data [11] from another perspective: the concomitant effect of 'not vaccinated' with the absence of any serious infectious disease in life. The two variables behave synergistically. Protection against melanoma is not only afforded by one or both of the vaccinations, but also by a history of one or more serious infectious diseases. In view of the heterogeneous aetiology of the infections, the influence on protection of individual infections cannot be reliably determined. Moreover, since these serious infections are uncommon nowadays, melanoma protection of the present European population is predominantly the result of prior vaccinations. Until 1975, more than 90% of the European population was vaccinated with vaccinia, but this is no longer the case in the younger population in which BCG vaccination is currently only given to a few. Accordingly, changes in vaccination strategies may be imposing an additional melanoma risk.

Table 1
Summary of the FEBIM study on the effects of vaccinia and BCG vaccination on the risk of melanoma development [11]

Vaccinations	Number of cases/number of controls	Adjusted Odds Ratios*	95% Confidence Intervals
Effect of vaccinations			
No vaccinia, no BCG	63/37	1.0	Reference
Vaccinia and BCG	271/341	0.41	0.25–0.67
Only BCG	19/26	0.40	0.18–0.85
Only vaccinia	250/223	0.60	0.36–0.99

FEBIM, FEBrile Infections and Melanoma Group; BCG, Bacille Calmette-Guérin.
* Adjusted for centre, sex, age, ethnic origin, freckling index, number of naevi and number of sunburns.

Table 2
Summary of the FEBIM study on the joint effects of 'not being vaccinated with vaccinia and/or BCG' on the risk of melanoma [11]

Co-variable	Vaccination with BCG and vaccinia		Concomitant effect (type of)
	Yes	No	
Serious infectious disease			
⩾1	1.00 *n* = 96	1.21 (0.67–2.06) *n* = 12	
0	1.12 (0.30–4.30) *n* = 516	3.03 (1.58–5.97) *n* = 88	Synergistic
	Vaccination with BCG or vaccinia		
	Yes	No	
Serious infectious disease			
⩾1	1.00 *n* = 98	1.97 (1.14–3.56) *n* = 12	
0	1.28 (0.35–4.90) *n* = 420	3.45 (1.79–6.80) *n* = 89	Synergistic

The Odds Ratios (95% Confidence Intervals) for melanoma risk and the number of patients and controls (*n*) analysed are shown. Odds ratios are adjusted for centre, sex, age, ethnic origin, freckling index, number of naevi and number of sunburns.

Table 3
Joint analyses of the melanoma risk indicator 'not being vaccinated with either BCG or vaccinia' compared with 'being vaccinated with BCG and/or vaccinia' and five co-variables of melanoma risk

Co-variable	Vaccination		Concomitant effect (type of)
	Yes	No	
Skin-type (Fitzpatrick)			
III/IV	1.00 *n* = 607	1.68 (0.93–3.05) *n* = 58	
II	1.80 (1.18–2.78) *n* = 396	2.15 (0.65–8.34) *n* = 30	
I	1.54 (1.16–2.05) *n* = 126	6.37 (3.50–19.64) *n* = 12	Synergistic
Sunburns in life			
0	1.00 *n* = 338	1.51 (0.76–3.01) *n* = 46	
1–5	0.93 (0.68–1.26) *n* = 596	2.29 (1.14–4.76) *n* = 45	
>5	1.39 (0.91–2.14) *n* = 191	5.30 (0.87–102.48) *n* = 8	Synergistic
Naevi			
0	1.00 *n* = 272	2.51 (1.16–5.77) *n* = 35	
1–4	1.05 (0.70–1.48) *n* = 387	5.24 (1.89–17.10) *n* = 22	
>4	1.56 (1.10–2.22) *n* = 465	1.71 (0.84–3.57) *n* = 42	Non-cumulative
Freckles on arm			
0	1.00 *n* = 457	3.26 (1.60–6.87) *n* = 39	
10–20	1.57 (1.17–2.10) *n* = 426	2.38 (1.09–5.32) *n* = 31	
>20	3.03 (2.13–4.35) *n* = 247	4.06 (1.78–10.17) *n* = 30	Non-cumulative
Nutritional selenium			
Sufficient	1.00 *n* = 1008	1.48 (0.94–2.06) *n* = 83	
Sub-optimal[a]	0.89 (0.62–1.29) *n* = 122	8.09 (1.84–35.55) *n* = 17	Uni-directionally enhancing

The Odds Ratios (95% Confidence Intervals) for melanoma risk and the number of patients and controls (*n*) analysed are shown. Odds ratios are adjusted for sex, age, and other known risk factors.

[a] Centres from the former West-Germany (Berlin, Göttingen, Hamburg).

Additional joint analyses between 'not vaccinated' and other co-variables reveal more concomitant effects which are of practical as well as theoretical relevance: a synergistic association with two indicators of skin damage, namely skin type Fitzpatrick I and >5 sunburns in life, and a non-cumulative association of the newly recognised melanoma risk factor 'not vaccinated' with, respectively, the two pigment anomalies, namely >4 naevi and >20 freckles on the arm (Table 3).

Thus, people with light sensitive skin and with repeated sunburns, who are already known to have a higher melanoma risk, are at an even greater risk if they have not been vaccinated. The observed concomitant effects of the vaccinations and the co-variables on melanoma risk imply that these factors are not independent. The non-cumulative association of the newly recognised risk indicator 'not vaccinated' with the pigment anomalies of more than 4 naevi and more than 20 freckles on an arm, respectively, indicates that 'not vaccinated' and the two variables are reflecting a single underlying melanoma risk factor. Moreover, this suggests that the target of the immune surveillance is an endogenous factor rather than one that is only expressed in cells that have already undergone malignant transformation.

From these data, it may be postulated that vaccinia and BCG vaccinations induce or enhance an immune surveillance mechanism that is able to suppress the manifestation of a genetically encoded melanoma risk factor. The association of this risk factor with two different types of pigment anomalies suggests that it may affect pigmentation and its regulation. The synergistic relationship of this risk factor with the two indicators of skin vulnerability, Fitzpatrick skin type I and more than 5 sunburns in life, indicates that it predisposes the skin to injury on exposure to ultraviolet (UV) radiation, particularly in patients with low level genetically-determined natural melanin pigment protection.

3. Is an immune surveillance mechanism for melanoma induced or enhanced by prior contact with pathogens exhibiting T cell epitopes that unexpectedly cross-react with a cellular 'melanoma risk marker'?

From the standpoint of the development of a hypothesis, three putative steps in the initiation of a melanoma may be considered, as summarised in Fig. 1.

1. In the initial step, a gene involved in the development of a melanoma is activated, and its gene products are expressed.
2. The subsequent events are determined by the activity of *immune surveillance* that is based on recognition of a 'marker peptide' closely linked to the 'melanoma risk protein' encoded on the 'melanoma gene'. The 'marker peptide' might be coded for by a very small open reading frame (ORF), one that is closely associated with the reading frame of the 'melanoma risk protein'. A 'marker peptide' of 8–11 amino acids would not depend on proteolytic processing before

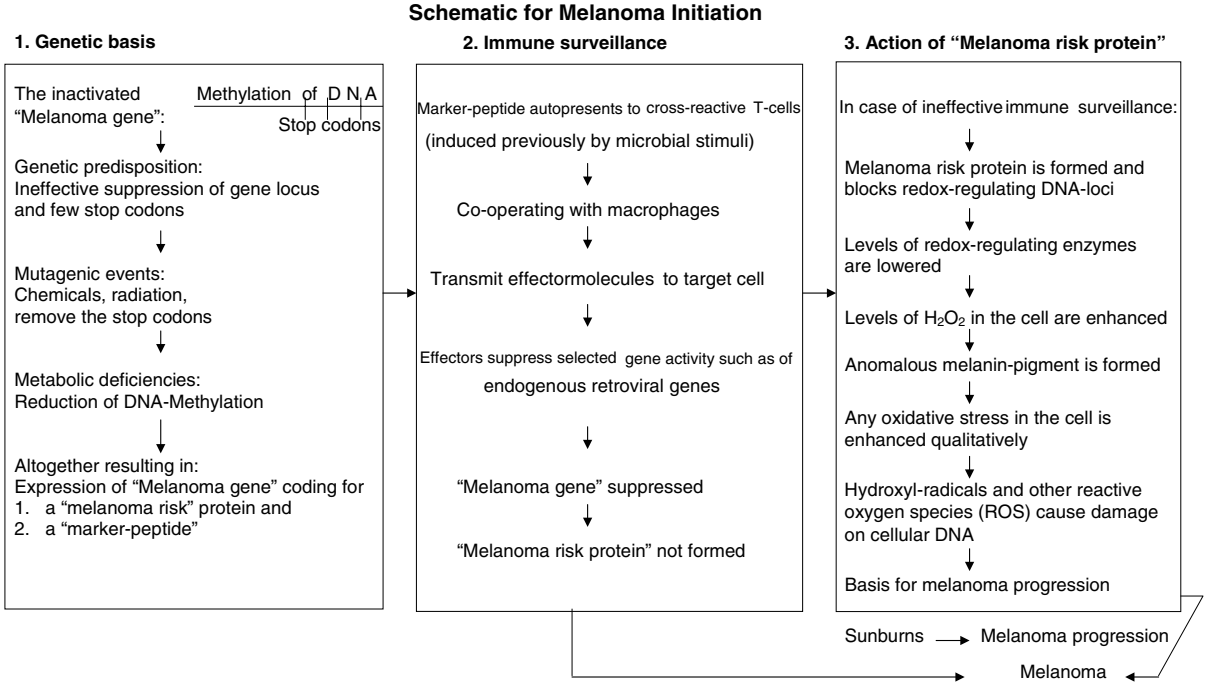

Fig. 1. Schematic for melanoma initiation.

presentation to the immune system by human leucocyte antigen (HLA) class 1 molecules. It is postulated that an effective melanoma immune surveillance based on recognition of this epitope depends on the induction of clones of unexpectedly cross-reactive $CD8^+$ T-cells by certain prior infections and/or vaccinations. Such T cells, through recognition of a 'marker peptide', might well be able to downregulate the cellular expression of a 'melanoma risk protein' or the activation of the gene coding for the 'marker peptide'.

3. If immune surveillance is not active or effective enough to inhibit the activity of the 'melanoma risk protein', this protein could be responsible for the disturbance of the redox-regulation within the cell, leading to the formation of an anomalous type of melanin which, in turn, leads to a qualitative enhancement of any form of oxidative stress within the cell. A high oxygen tension within the cell inhibits apoptosis. Thus, if the immune surveillance mechanism fails, the stage of tumour initiation gives way to that of tumour progression. At this stage, tumour progression induced, for example, by sunburns, is accomplished by the catalytic generation of highly reactive radicals and other reactive oxygen species (ROS) which damage the DNA within the cell. In the following sections, the diverse aspects of the concept of immune surveillance and the various factors involved are discussed.

4. Is a specific candidate target, sharing sequence homologies with certain microbial antigens, able to elicit an immune surveillance mechanism when presented to the immune system?

Over millions of years of evolution, DNA of retroviral origin acquired by infection has been incorporated into the genomes of human beings and their ancestors. These genetic elements, termed human endogenous retroviruses (HERVs), make up approximately 8% of the human genome and possibly outnumber the functional genes. They are thought to have played a key role in evolution by enlarging the genome and by contributing to genetic polymorphisms, including those of the complement proteins and the major histocompatibility complex [13].

Hundreds of HERVs have been described and allocated to several groups. Most are functionally degenerate, although some code for antigenic peptides and, rarely, whole retroviral proteins and enzymes or even viral particles, although no HERV capable of being transactivated into an infectiously transmissible virus has been found. In general, the 'younger' the HERV, the more likely it is to have some functional activity. The HERV-K group is of relatively recent origin, having been acquired around the time of the human–chimpanzee evolutionary split. By means of their transposon-like activity, HERVs are able to affect the expression of nearby genes, and they have therefore attracted great interest as oncogenes and as triggers for certain autoimmune disorders. Many HERV-encoded tumour marker antigens have been found on solid tumours and leukaemia cell lines, leading to speculations about their causative role in cancer.

Investigation of a specific cytotoxic immune response to melanoma cells in a patient with this disease led to the characterisation of the target antigen, a nona- or decapeptide with the amino-acid sequences of, respectively, MLAVISCAV and AMLAVISCAV, coded for by a human endogenous retrovirus in the HERV-K group [14]. This viral marker is termed HERV-K-MEL and is a putative key factor in melanoma development as it is present in most melanomas and also in their presumed precursors, the dyplastic melanocytic naevi, but not in normal skin or in other normal tissue. Accordingly, the expression of this specific peptide may, directly or indirectly, be associated with cell damage paving the way for the development of malignancy. The presentation of this peptide to $CD8^+$ T cells is HLA-restricted, being presented in association with the HLA class I-molecule HLA-A2 which has a frequency of approximately 50% in the European population [14]. Since HERV-K-MEL is a nona- or decapeptide, its presentation to the immune system with HLA-class I does not depend on proteolytic antigen processing.

The above considerations raise the question of why various vaccinations and natural infections confer protection against melanoma and how this is related to the surveillance mechanism. This, as discussed above, we ascribe to exposure to pathogens, by natural infection or immunisation, with unexpected antigenic cross-reactivity and the resulting expanded populations of cross-reactive T cells [15]. Similarities could be detected between the HERV-K-MEL antigen and peptide sequences in over 70 human pathogens, as shown in Table 4. The short peptide sequences from these microorganisms, many of them able to cause severe infectious disease, are, at least in principle, capable of being presented as small peptides by HLA-A2 molecules because of the presence of known anchor sequences for the HLA-A2 molecule. Prior vaccinations against, or infections by, one or more of these microorganisms might well lead to clonal expansion of cells able to participate in immune surveillance mechanisms involving cells presenting the HERV-K-MEL peptide.

It is noteworthy that the microorganisms found to contain sequence homologies with the HERV-K-MEL peptide include the causative organisms of most of the infectious diseases that were found to induce protection against melanoma in the FEBIM study, whereas

Table 4
Peptide sequences from selected examples of pathogenic microorganisms exhibiting homologies to the (deca)/nonapeptide (A)MLAVISCAV or (A)MLAVVSCAV from HERV-K-MEL with conserved anchor sequences (in bold) for presentation to $CD8^+$ cells by HLA-A2 class I molecules[a]

Microorganism	Consensus sequence	Sequence	Length	Position	Polypeptide
Bacillus cereus	**ML**_AVIS.**AV**	MLGAVIS_AV	338	160–168	Ferric anguibactin transport system permease protein fatD
Bacteroides thetaiotaomicron	**ML**.++SC**A**	MLVLLSCA	257	1–8	Conserved hypothetical protein
Campylobacter jejuni	**+**LAVI.**AV**	LLAVI—AV	488	243–249	Putative amino-acid transport protein
Chlamydia trachomatis	**AM**.+A++SC**AV**	AMFVAIVSCAV	360	137–147	Hypothetical protein
	M+..ISC**AV**	MMDAISCAV	412	248–256	Glutamate symport
Entamoeba histolytica	**AML**.V.SC**A**	AMLVVSSCA	843	553–561	19 S Cap proteasome S2 subunit
Enterococcus faecalis V583	**A.L**.+ISC**A**	ATLNIISCA	324	289–297	Ribose-phosphate pyrophosphokinase
Enterococcus faecium	**A+L**AVI.C**A+**	AVLAVI_CAI	450	408–416	Hypothetical protein
Escherichia coli	**AML**A_VVS.**AV**	AMLAVVSGAV	156	22–32	Cytochrome *c*-type protein
	+LA+.SC**AV**	LLAIASCAV	191	115–120	Putative fimbrial-like protein sfmA
Giardia lamblia	A++AVI.C**AV**	AVIAVIGCAV	569	545–554	GLP_77_40692_38983
Haemophilus influenzae	**LA**.VSC.**V**	LAGVSCDV	610	318–325	Glucosamine-fructose-6-phosphate aminotransferase
Klebsiella pneumoniae	A.**LA**+I.C**A**	AFLAMIPCA	540	179–187	PTS system, α-glucoside-specific II BC component
	L+.ISC**AV**	LSGISCAV	373	113–120	Hypothetical 42.6 kDa protein in CPS region (ORF8)
Legionella pneumophila	A**+L**A+.S.**AV**	AVLALGSSAV	289	10–19	Major outer membrane protein precursor
Leptospira interrogans	**ML**..VSC.**V**	MLGFVSCIV	804	441–449	Predicted HD family protein
Listeria monocytogenes	**AML**A.—IC**A**	AMLAAIDYFCA	365	105–115	Similar to aminotripeptidase
Moraxella catarrhalis	**LAVI**+.**AV**	LAVIA—AV	453	8–14	Outer membrane protein CD
Mycobacterium bovis (including BCG)	**+LAV_V..AV**	LLAVDVVPIAV	330	70–80	Probable periplasmic iron-transport lipoprotein
Mycobacterium fortuitum-chelonae	**+**L.VV..**AV**	LLGVV_AV	409	389–395	Tap protein
Mycobacterium leprae	**AML**A+.+**AV**	AMLAIFAAV	300	205–212	CDP-diacylglycerol-serine *o*-phosphatidyltransferase homologue
Mycobacterium marinum	**AM**.AV.+.**AV**	AMSAVAALAV	309	12–21	Erp protein precursor
Mycobacterium tuberculosis	**M+**A+IS.**AV**	MIALISYAV	608	165–173	Hypothetical protein
Neisseria gonorrhoeae	**LA**.VS.**AV**	LAGVSYAV	174	130–137	Outer membrane protein
Neisseria meningitides	**ML**..I+C**A**	MLGGITCA	823	96–103	Cation transport ATPase, E1–E2 family
Orf virus	**+L**AV.+.**AV**	LLAVAAVAV	74	38–46	Homolog to vaccinia virus F9L protein
Pasteurella multocida	**LAV**++C**A**	LAVVTCA	443	55–61	Unknown
Pseudomonas aeruginosa	**AML**.+IS.**AV**	AMLVIIS_AV	296	273–281	Conserved hypothetical protein
Salmonella enterica	**ML**A+I..**AV**	MLAMIVSAV	710	662–670	Putative membrane protein igaA homolog
Serratia marcescens	A.**LAV**+.C**AV**	AFLAVVHCAV	217	182–191	Unknown
Shigella flexneri	**AML**A.V+S.**AV**	AMLALVVSGAV	156	22–32	Cytochrome *c*-type protein
Staphylococcus aureus	**ML**A.IS.**AV**	MLAGISVAV	331	244–252	Lipoprotein
Streptococcus agalactiae	**A+L**.A.ISC**AV**	ALLRAFISCAV	431	159–169	Sensor histidine kinase
Streptococcus pneumoniae	**+**LAVV+—C**AV**	LLAVVTIVFCAV	136	22–33	Conserved hypothetical protein
Streptococcus pyogenes	**ML**—AV.SC**A+**	MLKQAV_SCAI	164	112–121	Conserved hypothetical protein
Treponema pallidum	**LA**V.+C**AV**	LAVSTCAV	1140	209–216	transcription-repair coupling factor
Vaccinia virus	A.**LAV**.I+C**A**	ASLAVVIACA	1504	116–125	Polyprotein
Vibrio cholerae	**LAV++CA**	LAVVTCA	442	55–61	Anaerobic C4-dicarboxylate transporter
Vibrio parahaemolyticus	**AML**A.I.C.**V**	AMLAAIMCIV	295	143–152	Putative transmembrane protein
Yellow fever virus	**LA**V.S.**AV**	LAV_SSAV	3411	3287–3293	Polyprotein
Yersinia enterocolitica	**L**.V++C**AV**	LNNVVACAV	95	68–75	Transposase

Also given are the polypeptides and the position of the sequence therein.
HLA, human leucocyte antigen; HERV, human endogenous retrovirus; ATP, adenosine triphosphate; CDP, cytidine-5′-diphosphate.

[a] Additional human pathogenic microorganisms with homology: *Ajellomyces capsulatus*, *Babesia bovis*, *Bacillus anthracis*, *Bacteroides fragilis*, *Bartonella bacilliformis*, *Bordetella pertussis*, *Bordetella bronchiseptica*, *Borrelia burgdorferi*, *Brucella mellitensis*, *B. abortus*, *B. suis*, Californian encephalitis virus, *Chlamydia pneumoniae*, *Clostridium tetani*, *Corynebacterium diphtheriae*, *Coxiella burnetii*, *Cryptococcus neoformans*, *Francisella tularensis*, *Haemophilus ducreyi*, *Helicobacter pylori*, Japanese encephalitis B virus, *Leishmania donovani*, *Leishmania major*, molluscum contagiosum, *Mycoplasma penetrans*, *Plasmodium falciparum*, *Pneumocystis carinii*, *Proteus vulgaris*, *Rochalimaea quintana*, *Rickettsia rickettsii*, *Salmonella typhi*, *Staphylococcus epidermidis*, tick-borne encephalitis virus, *Toxoplasma gondii*, *Trichomonas vaginalis*, *Tropheryma whippeli*, *Trypanosoma brucei gambiense*, *Trypanosoma cruzi*, Venezuelan equine encephalitis virus, and *Yersinia pestis.*

Table 5
Vaccinations and diseases (with fever of >38.5 °C) which correlate significantly with reduced melanoma risk

Vaccination or disease	OR	95% CI	Causative agents with the homology	Causative agents without the homology	Related microorganisms with the same homology
BCG-vaccination	0.40	0.18–0.85	*Mycobacterium bovis*		*Mycobacterium tuberculosis*, *Mycobacterium leprae*, *Mycobacterium avium-intracellulare*, *Mycobacterium fortuitum-chelonae*
Vaccinia-vaccination	0.60	0.36–0.99	Vaccinia virus		orf virus, molluscum contagiosum virus, ectromelia virus, African swine fever virus
Tuberculosis of the lung	0.16	0.01–0.98	*Mycobacterium tuberculosis*, *Mycobacterium bovis*		
Infections due to *Staphylococcus aureus*	0.54	0.31–0.94	*Staphylococcus aureus*		*Staphylococcus epidermidis*
Sepsis	0.23	0.06–0.70	(a) Frequent causes: *Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Klebsiella pneumoniae*, *Enterococcus faecalis*, *Enterococcus faecium*, *Pseudomonas aeruginosa*, *Bacteroides thetaiotaomicron*, *Bacteroides fragilis*, *Neisseria meningitides*, *Streptococcus agalactiae*, *Escherichia coli*, *Proteus vulgaris*. (b) Rare causes: *Haemophilus influenzae*, *Bacteroides fragilis*, *Salmonella enteritidis*, *Pasteurella multocida*, *Neisseria gonorrhoeae*, *Campylobacter jejuni*, *Serratia marcescens*		
Pneumonia	0.45	0.27–0.73	(a) Frequent causes: *Streptococcus pneumoniae*, *Staphylococcus aureus*, *Chlamydia trachomatis*, *Streptococcus pyogenes*, *Haemophilus influenzae*, *Klebsiella pneumoniae*, *Neisseria meningitides*, *Pseudomonas aeruginosa*, *Bacteroides thetaiotaomicron*, *Bacteroides fragilis*, *Chlamydia pneumoniae*, *Legionella pneumophila*, respiratory syncytial virus. (b) Rare causes: *Coxiella burnetii*, *Ajellomyces capsulatae*, *Bacillus anthracis*, *Moraxella catarrhalis*, *Leptospira interrogans*, *Pneumocystis carinii*, *Francisella tularensis*, *Cryptococcus neoformans*, *Mycobacterium avium-intracellulare*		
Influenza	0.65	0.48–0.86	*Haemophilus influenzae*, *Streptococcus pneumoniae*, *Staphylococcus aureus*	Influenza virus[b]	
Infectious enteritis[a]	0.70	0.52–0.94	*Shigella dysenteriae*, *Salmonella enteritidis*, *Helicobacter pylori*, *Campylobacter jejuni*, *Yersinia enterocolitica*, *Escherichia coli*, *Clostridium difficile*, *Vibrio parahaemolyticus*, *Entamoeba histolytica*, *Bacillus cereus*, *Staphylococcus aureus*, *Yersinia enterocolitica*, *Pseudomonas aeruginosa*, *Entamoeba histolytica*, *Vibrio cholerae*, *Giardia lamblia*,		

The table shows frequent and rare causative agents of these diseases with sequence homologies to the appointed target antigen HERV-K-MEL (including the anchor sequences for presentation with HLA-A2) as well as frequent causes of the diseases without the sequence homology.
OR, Odds Ratio; 95% CI, 95% Confidence Interval. Odds ratios are adjusted for centre, sex, age, ethnic origin, freckling index, number of naevi and number of sunburns.
[a] With at least elevated temperature.
[b] Severe disease associated with influenza is often the result of bacterial superinfection.

Table 6
Diseases (with fever of >38.5 °C) which did not correlate significantly with reduced melanoma risk

Vaccination or disease	OR	95% CI	Causative agents with the homology	Causative agents without the homology
Bronchitis	0.56	0.29–1.05	*Streptococcus pneumoniae*, *Haemophilus influenzae*, *Moraxella catarrhalis*, *Chlamydia trachomatis*, *Bordetella pertussis*	Diverse respiratory viruses *Mycoplasma pneumoniae*
Meningitis	0.59	0.20–1.59	*Neisseria meningitides*, *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Streptococcus agalactiae*, *Enterococcus faecium*, *Enterococcus faecalis*, *Listeria monocytogenes*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Salmonella enteritidis*, *Staphylococcus aureus*	Diverse viruses such as enteroviruses, mumps virus, adenovirus, EBV
Hepatitis	0.74	0.35–1.53		Diverse hepatitis viruses, EBV
Herpes simplex	0.88	0.42–1.81		Herpes simplex virus
Erysipelas	0.77	0.32–1.82	*Streptococcus pyogenes*	Other *Streptococci*
Rheumatic fever	0.84	0.23–2.00	*Streptococcus pyogenes*	Other *Streptococci*
Urinary tract infections	0.86	0.41–1.79	*Klebsiella pneumoniae*, *Enterococcus faecium*, *Enterococcus faecalis*, *Pseudomonas aeruginosa*, *Serratia marcescens*	B-Streptococci, *Staphylococcus saprophyticus*, *Providentia*, *Alcaligenes*
Cholecystitis	0.95	0.32–2.78	*Streptococcus pyogenes*, *Bacteroides*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Salmonella enteritidis*	Other *Streptococci*

The table shows frequent and rare causative agents of these diseases with good sequence homology to the appointed target antigen HERV-K-MEL (including the anchor sequences for presentation with HLA-A2) and frequent causes of the diseases without the sequence homology. Odds ratios are adjusted for centre, sex, age, ethnic origin, freckling index, number of naevi and number of sunburns.
EBV, Epstein Barr Virus.

infections which were not significantly associated with melanoma protection were mainly caused by other microorganisms (Tables 5 and 6).

At the present time, it is not known whether the relevant microbial peptides are cleaved from corresponding proteins by proteolysis. It also remains to be determined whether only those individuals who express the HLA-A2 tissue type are protected against melanoma by vaccination or serious infectious disease. It is possible that other as yet undiscovered melanoma-associated antigens show different forms of HLA restriction. Thus, for example, melan A, a DNA repair enzyme, is another important melanoma antigen. The human gene for melan A seems to have been acquired from prokaryotes by horizontal gene transfer and, as it shares sequence homologies with analogous proteins in most bacteria [16], this target (as well as others) might therefore substitute for the HERV-K-MEL 'marker peptide' of melanoma risk.

Although most other types of tumour that have been studied differ from melanoma in not expressing the HERV-K-MEL peptide, there are exceptions including some sarcomas and carcinomas [14]. Accordingly, this type of immune control of tumours may not be restricted to melanoma, especially if the many other described HERV-related cancer antigens elicit similar or identical immune responses.

5. Are human endogenous retroviruses the basis of the endogenous melanoma risk factor?

Endogenous retroviral genes are normally not expressed in melanocytes or other normal adult cells, although it has recently been shown that the HERV-K env-protein (envelope protein) is formed in melanoma [17]. The corresponding gene is, with 60–200 copies, part of the human genome [18,19]. The HERV-K-MEL peptide stems from the env-gene region of HERV-K, though it is not part of the same ORF that codes for the env-protein. A genetic pre-disposition to melanoma would result, for example, from the absence of stop codons within at least one copy of a gene, such as the env-gene, and from a failure of gene repression which can result from a hypomethylation of genomic retroviral DNA (see below). Accumulating mutagenic events in a specific somatic cell removing all stop codons of this gene in combination with a failure of gene repression could launch the initiating events in the development of melanoma.

Despite widely held earlier views, the antigens recognised by the immune system are not 'foreign', but 'weak-self', with immune regulatory pathways preventing weak self-recognition from inducing autoimmune reactions. Such self-recognition, which develops during embryogenesis (during which time antigens rarely expressed in the adult are expressed and recognised), enables the immune system to maintain a surveillance of the body to detect and rectify harmful endogenous events such as malignant transformation as well as the effects of invading pathogens. The internal self-image of the body 'seen' by the immune system has been termed the 'immunological homunculus' [20]. This is a paradigmatic change in immunology. It allows for an easier understanding why the HERV-related antigens are part of 'self' and, as such, are detectable, by immune surveillance mechanisms when expressed on normal or cancer cells. T cell clones expanded by prior immune contact with cross-

reactive microorganisms are the initiators, or at least the enhancers, of the immune surveillance. We postulate that this immune surveillance potentially affects other retroviral genes, either their expression or functional activity, as well as the expression of other cellular genes which come under the influence of endogenous retroviral elements [13,19].

6. Does the target-specific immune surveillance suppress endogenous retroviral activity?

As mentioned above, the HERV-K-MEL-associated antigen mediates a cytotoxic immune response and, indeed, such cytotoxicity led to its discovery. While cytotoxicity undoubtedly plays a key role in the immune defence against established tumours, we postulate that cytolytic immune reactions are not involved in the prevention of the initial stages of melanoma development – that part of the story which is of relevance with regard to our current hypothesis on melanoma prevention. Rather than destroying cells that present the auto-antigen, it appears more likely that the immune defence mechanisms suppress its expression and also the expression of the closely associated HERV-K env-protein. Thus, a principal function of immune surveillance may well be to rectify an underlying malfunction, thereby preventing the transcription of the HERV-K-MEL pseudogene as well as of the HERV-K env-gene and possibly to also preventing the adverse effects of the expression of other retroviral genetic elements. There are clear advantages to the host of suppressive rather than cytotoxic immune reactions against endogenous antigens which are coded for in all of the cells of the body.

7. How is a suppressive immune response achieved?

$CD8^+$ T cells expanded by prior contacts with microorganisms with cross-reactive antigens should, as described above, recognise the HERV-K-MEL retroviral antigen which is presented with HLA-A2. When acting as specific suppressor T cells, they transmit one or more soluble factors to the target cells by cell-to-cell contact. The involvement of such soluble factors in the mode of action of T suppressor cells has long been recognised and a major candidate is the ganglioside, LM1 [21]. This molecule has been shown in several studies to have important effects on cell growth and differentiation – it can normalise a malignant phenotype in various pre-malignant cell lines, cause a cell cycle arrest within the G0/G1 phase of the cycle [22,23], and it suppresses the expression of retroviral mRNA (see below).

In this context, it has been suggested that the signal transduction of the LM1-effect involves phospholipase A2 which is regulated by G-proteins and mitogen activated protein (MAP)-kinases, and via an activation of protein kinase C. Various gene activities induced by LM1 have been demonstrated in human promyelocytic cells [24], and three of the differently expressed mRNAs identified as stemming from parts of the human genome have recently been characterised (Nos. 7, 10 and 11 in Table 1 of [24]; Krone, data unpublished). Thus, LM1 induced an mRNA coding for the hypothetic protein MGC22679 with motifs known to mediate transcriptional repression [25], whereas it suppressed two mRNAs from the human genome which are, respectively, highly homologous to human endogenous retroviruses; namely, HERV-K (I) and a human cellular counterpart of feline sarcoma retrovirus mRNA. The gene product of the latter has transforming capabilities, and its activity is required for maintenance of cellular transformation [26]. The mRNAs of the two aforementioned retroviruses themselves were not found but, instead, a 'patchwork' of the retroviral sequences making up >95% of these mRNAs was found, suggesting a suppression of retroviral gene expression. In addition, HERV expression is known to be strongly downregulated by agents that induce cellular differentiation [13]. The influence, postulated above, on the expression of proteins needed for normal redox processes was also observed. In the same experiment, LM1 induced NADH ubiquinone oxidoreductase and a thioredoxin homologue, an enzyme and a protein co-enzyme, respectively, that are physiologically involved in redox processes [24].

One important reason why endogenous retroviral genes are not normally expressed by cells is that transcription of the HERV provirus is prevented by hypermethylation of the DNA [27,28]. Conversely, expression of these antigens in some cells is due to DNA hypomethylation. In such cells, a likely reason why transmission of LM1 stemming from appropriately co-operating macrophages suppresses expression of the gene is that LM1 induces S-adenosyl-homocysteine hydrolase, the enzyme which catalyses the production of active methyl groups for the methylation of DNA [24,29], thereby re-establishing hypermethylation.

Although we could not find any specific report in the literature of a role for LM1 in the regulation of melanomas, such a role is, in principle, probable. As has also been described for other tumour cells, various gangliosides are known to be enriched on melanomas, with the ganglioside pattern differing, qualitatively and quantitatively, from that on precursor cells [30], and gangliosides shed from melanomas are known to have immunosuppressive activity [31]. Some gangliosides, such as GD1b, GT1b and GQ1b, have been shown to suppress the growth of human melanoma [32]. Moreover, a role in signal transduction has been ascribed for gangliosides [33].

Specific cytotoxic immune responses to the expressed HERV may also be directly suppressed by the antigen itself [34], or they could be indirectly inhibited by

suppressor macrophages [35]. A major problem is that different immune reactions may be required for the prevention of cancer and for the destruction of established tumours. In this context, the T-helper cell type 2 cytokine interleukin 4 enhances the antigenicity of melanoma cells, thereby increasing their ability to activate cytotoxic T cells and to act as targets for these cells [36].

8. What directs immune surveillance towards suppressive signalling?

First, within the framework of the above-mentioned concept of 'weak self-recognition', T cell clones with a close homology to the endogenous target antigen should have an intrinsic preference for a suppressive mode of action. Second, it has long been recognised that exposure to a small amount of antigen favours induction of tolerance, but why? The simplest idea is that a specific T-cell requires repeated contact with an antigen to become activated. Several possible mechanistic models are reviewed by Jun and Goodnow [37]. We suggest that this activation is based on the induction of receptor cross-linking which cross-activates adjacent kinases, as demonstrated for receptor tyrosine kinases, such as the insulin receptor [38]. After repeated antigen contacts, and with the help of active $CD4^+$ T-cells, the specific $CD8^+$ T-cell becomes activated to have a cytotoxic effect on an antigen-presenting cell. On the other hand, in the presence of a relatively small amount of antigen, and in the absence of the specific help of active $CD4^+$ T-cells and of professional antigen-presenting cells, the specific $CD8^+$ T-cell is likely to encounter the antigen just once, rather than repeatedly over a short period of time. (A comparable situation is given in the case of a slowly increasing amount of antigen, together with a slowly enlarging population of specific T-cells.) Although only in contact with the antigen once, the T-cell will have repeated contacts with cells of granulocyte/macrophage lineage. We postulate that this is a physiological process and that during these contacts with cells of this lineage, such T-cells will acquire – from the cell membrane of the granulocytes/macrophages – gangliosides and other lipids which modify the structure of the T-cell receptor complex. Upon contact with their specific target, these T-cells will, as a result of the modification of their receptors, react in a tolerogenic and immunosuppressive manner, as described for the insulin receptor tyrosine kinase activity that is blocked by the ganglioside, LM1 [39].

9. How is active immune surveillance maintained throughout life?

Most human beings have melanocytic naevi. Although dysplastic naevi are regarded as precancerous lesions, only a few will develop into melanoma, indicating that although most persons are adequately protected, some require enhancement of their protection. In this context, it is possible to distinguish four groups of individuals in relation to melanoma –

Group 1.
Persons who are adequately protected by immune surveillance and never develop a melanoma.
Group 2.
Those who have some degree of protection and develop melanoma late in life.
Group 3.
Those who are less well protected and develop melanoma earlier in life.
Group 4.
Those who have a different type of melanoma that is not subject to the immunological control under consideration and who tend to develop the disease early in life.

It is likely that many persons in groups 2 and 3 move to group 1 after experiencing appropriate immune stimuli, but those in group 4 are unlikely to be protected and develop melanoma, irrespective of their history of vaccination or infections. Thus, in a vaccinated population, relatively more melanoma patients would be expected to belong to group 4. This could explain the paradoxical findings in the FEBIM studies [11] that, although the incidence of melanoma was significantly lower in individuals with a history of vaccinia and/or BCG vaccination than in those who had received neither vaccination, the mean age of those developing the disease was about 10 years lower in the former population compared with the latter ($P < 0.0001$, t-test).

10. Does the 'melanoma risk protein' disturb the cellular redox regulation, thereby inhibiting apoptosis?

It is postulated that the HERV-K env-protein is the principal candidate for the 'melanoma risk protein' and, accordingly, the complete env-protein or truncated fragments of it play an important role in melanoma development. The env-protein has, between amino acids 488 and 529, a sequence homology with a human nuclear factor termed the Oxygen Responsive Element Binding Protein (OREBP), also known as the Nuclear Factor of Activated T Cells (NFAT) [40,41]. This factor is essential for controlling the redox regulation of the cell and is itself regulated by the oxygen tension within the cell. OREBP is a homo-dimer that forms a stable dimer with human DNA at the two oxygen responsive elements, ORE1 and ORE2. The homologous viral env-protein might block the binding of OREBP to DNA, altering the redox regulation of the cell via the biosynthesis of an anomalous melanin pigment.

OREBP regulates the expression of glutathione peroxidase, a selenium-dependent enzyme, that catalyses the reduction of hydrogen peroxide (H_2O_2) to water (H_2O). Low levels of the enzyme lead to enhanced concentrations of H_2O_2 and of superoxide anion radicals. Moreover, melanin binds metal ions. In the presence of elevated levels of H_2O_2, Fe^{2+}-ions catalyse the Fenton reaction so that hydroxyl-radicals ($^{\cdot}OH$) are formed. Within the cell, an accumulation of metal ions is associated with metallothioneins, elevated intracellular levels of which have recently been found to be associated with an unfavourable prognosis for melanoma [42]. Accordingly an enhanced level of metallothioneins in a premelanotic cell might well be an unfavourable risk factor for melanoma. A well regulated redox condition of the cell is necessary for the biosynthesis of normal melanin, whereas an anomalous pigment is formed in the presence of redox dysregulation. Endogenous or exogenous oxidative stress is enhanced by the anomalous pigment, and qualitatively more reactive oxygen species, such as $^{\cdot}OH$, are formed [43]. These cause damage to diverse cellular structures, including DNA, and the enhanced oxygen tension induces the activation of the nuclear factor NF-κB and of other redox-dependent transcriptional factors that have an anti-apoptotic activity [44–46], together paving the way to malignancy. This process may continue for years or decades.

In this context, there may be a parallel to nutrition-derived selenium which exerts an influence on these processes. Low levels of this element have been found in adults with a number of cancers, notably breast cancer [47], and in children, particularly those with widespread cancer [48]. Conversely, although questions of cause and effect remain, optimal levels of selenium are associated with tumour protection as well as with a late onset of tumour manifestation. Selenium levels in water supplies and food vary considerably between and within countries. Using recently published data [49], we have shown, for the first time, that low selenium levels in the local environment are associated with an enhancement of melanoma risk in the 'not vaccinated' group, but not in the vaccinated group (Table 3). This suggests that the underlying melanoma risk factor manifests itself in a selenium-dependent way, possibly related to the role of the selenium-dependent enzyme, glutathione peroxidase.

In the FEBIM study, patients in the combined centres Berlin and Göttingen, Germany, lived in regions with low selenium concentration in the soil. Healthy control persons from Berlin (Germany) had a median selenium level of 0.452 μg/g in their toenails which was lower than that in all the other seven European countries investigated and in Israel, with a median selenium level of 0.611 μg/ml [50]. As our findings suggest that the vaccinia and BCG vaccinations negate a melanoma-enhancing effect of a suboptimal nutritional selenium supply, further studies on selenium and tumour risk should take the vaccination status into account.

11. Statement and summary of the hypothesis

We postulate that BCG and vaccinia as well as certain common causes of infectious disease are associated with a reduced risk of melanoma because they code for antigenic determinants showing sequence homologies with the HERV-K-MEL-antigen, a product of a pseudo-gene which is part of the env-gene of the endogenous human retrovirus K (HERV-K). A suppressive immune reaction appears to inhibit the expression of endogenous retroviral genes, such as the HERV-K env-gene, that might otherwise result in malignant transformation years or even decades later. The HERV-K env-protein has homologous amino acid sequences with the human nuclear factor OREBP which controls the expression of glutathione peroxidase. The former might, in turn, suppress the formation of this and other redox-enzymes, of which appropriate levels are necessary for a normal redox potential within the cell.

12. Are there realistic vaccination strategies for the prevention of melanoma?

Certain microorganisms have been used from time to time as non-specific immune stimulants in the treatment of cancer. These include BCG and listeria, both of which have sequence homologies with the HERV-K-MEL antigen (Table 4). Among the various microorganisms with this homology, *S. marcescens* shows a particularly close sequence homology with the melanoma antigen – only *Entamoeba histolytica* and *Treponema pallidum* show a closer homology. In this context, killed *S. marcescens* (previously termed *Bacillus prodigiosus*) was a principal component of Coley toxins used in the late 19th and early 20th centuries for the treatment of sarcomas and other malignancies, including melanoma. In view of this hypothesis, Coley toxins or *S. marcescens* in some other suitable formulation might well be worth a reinvestigation for use in cancer prophylaxis, as well as in treatment.

Vaccinia and BCG were once widely used as vaccines. The former was abandoned with the conquest of smallpox, and BCG is no longer used in many developed nations. More than a dozen of the other microorganisms with sequence homologies with the HERV-K-MEL antigen are used to prepare vaccines. Among these, the most frequently administered are tetanus and pertussis vaccines, but the currently used vaccine formulations (tetanus toxoid and acellular pertussis vaccine) do not contain the relevant antigens. Moreover, most of the non-viable vaccines are in formulations that induce

antibody responses rather than cell-mediated immunity. Several relevant currently available vaccines are viable; namely one each against yellow fever, a new Japanese encephalitis B, and Venezuelan equine encephalitis viruses, and an oral vaccine against typhoid fever. It remains to be determined whether these live vaccines can deliver co-stimulatory signals necessary for the induction of the mechanisms protecting against melanoma. Despite the possible suitability of these vaccines, the strongest candidate is BCG as there are other pressing arguments in favour of its re-introduction, notably the resurgence of tuberculosis, including multi-drug resistant forms, in developed as well as in developing nations [51].

13. Are there evolutionary processes underlying HERV-K-MEL-mediated tumour protection?

We suggest that the homology to the HERV-K-MEL found in so many pathogens (which also implies that there are homologies between the pathogens) did not come about by chance, but because of related evolutionary pressures. It seems to be beneficial to the microorganism, as well as to the host, in that the latter has a chance to attack the microorganism immediately after an encounter because of cross-reactive memory T-cells which resulted from prior immunologically effective contacts with unrelated but epitope-homologous pathogens. On the other hand, some microrganisms, notably intracellular pathogens, can establish a persistent, although often latent, infection. When such a microorganism has invaded a cell, this cell presents the homologous peptide epitope, resulting in the immediate and effective suppressive immune reaction against this cell, as discussed above. This would affect not only the activity of the cell, but would also result in a downregulation of the metabolism and replication of the microorganism. As a consequence, only small amounts of microbial antigen would be encountered by cells of the immune system, leading to the generation of tolerance to the larger amounts of antigen released later in the active disease process.

14. Relevance of the hypothesis to the overall maturation and function of the immune system

In recent years, it has become apparent that the normal maturation of the immune system, especially during the first few years of life, is critically dependent on environmental factors. Over millennia, the human immune system has evolved to 'expect' certain infections and other immunological challenges that drive its maturation [52,53]. In the industrially developed nations, there has been, over the past few decades, a significant increase in the incidence of a number of diseases that are, at least to some extent, the result of dysregulated immune responses. Such conditions include allergic and atopic disorders, a wide range of autoimmune diseases, and cancer. The rising incidence of these diseases has been attributed to environmental changes that have prevented contact of the human population with the usual antigenic stimuli required for an adequate maturation of the immune system [52,53].

Silent HERVs can be reactivated by environmental conditions that induce cellular stress including physical or chemical agents and DNA viruses [19]. In this context, it is noteworthy that two major classes of disease – cancer and autoimmune disorders – have been linked to changing environmental conditions associated with expression of HERVs. It is thus probable that deficits in maturation of the immune system compromise the normal regulatory mechanisms that prevent the transactivation of HERV pseudo-genes and the ensuing associated disease processes. The challenge to immunologists is to characterise the exceedingly complex immunoregulatory pathways and the cellular and cytokine markers that indicate immune dysregulation and that could be used to monitor its therapeutic correction.

Many studies commencing in the late 1950s have shown that T cell-cross-reactivity between non-related (heterologous) microorganisms has an important influence on T cell immunodominance and maintenance of memory [15]. This phenomenon has been given the name 'Original antigenic sin' [54]. We postulate here that cross-reactivity also extends to HERV-encoded 'self'-antigens and that this may well explain the lasting influence of previous vaccinations and infections on the induction of $CD8^+$ T cell responses against subsequent processes contributing to malignancy. Much attention has recently been devoted to changes in the balance between Th1 and Th2 T cell populations and their respective cytokines in cancer as well as the impact of successful therapy on this balance [55–58]. However, these changes may merely reflect in a superficial manner the activity of a far more complex underlying immunoregulatory network involving the interplay of various regulatory T cell (Treg) populations [52]. The immunoregulatory network is also affected by endocrine factors, notably the balance between dehydroepiandrosterone and corticosteroids within tissues and lesions [59], and in this context, there is evidence that mRNA expression of some HERVs in various normal and tumour tissues is profoundly influenced by steroid hormone balances [60].

A detailed elucidation of the complex immune processes involved in the prevention of malignant transformation leading to melanoma, leukaemia and other malignancies, as well as to the destruction of established malignant cells, will require an enormous amount of work. The hypothesis presented here may prove to be helpful. Furthermore, there is now considerable empiri-

cal evidence that environmental factors exert a major impact on the prevalence of several cancers, as they do on autoimmune, atopic and allergic disorders. There are also clear indications for a beneficial impact of simple vaccination strategies on human health far beyond the specific illnesses that they were intended to combat [58,61]. National and international agencies should therefore give serious and urgent consideration to a revision of their vaccination policies.

Conflict of interest statement

None declared.

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References

1. Grange JM, Stanford JL, Stanford CA. Campbell De Morgan's 'Observations on cancer', and their relevance today. *J Roy Soc Med* 2002, **95**, 296–299.
2. Coley WB. The treatment of malignant tumors by repeated inoculations of erysipelas. Historical article of 1882, reprinted in *Clin Orthop* 1991, **262**, 3–11.
3. Malmstrom PU, Wijkstrom H, Lundholm C, Wester K, Busch C, Norlen BJ. 5-year follow up of a randomized prospective study comparing mitomycin C and bacillus Calmette-Guérin in patients with superficial bladder carcinoma. Swedish-Norwegian Bladder Cancer Study Group. *J Urol* 1999, **161**, 1124–1127.
4. Czarnetzki BM, Macher E, Suciu S, Thomas D, Steerenberg PA, Rumke P. Long term adjuvant immunotherapy in stage I high risk malignant melanoma, comparing two BCG preparations versus non-treatment in a randomized multicentre study (EORTC protocol 18781). *Eur J Cancer* 1993, **29A**, 1237–1242.
5. Grange JM, Stanford JL. BCG vaccination and cancer. *Tubercle* 1990, **71**, 61–64.
6. Perrillat F, Clavel J, Auclerc MF, *et al*. Day-care, early common infections and childhood acute leukaemia: a multicentre French case-control study. *Br J Cancer* 2002, **86**, 1064–1069.
7. Ma X, Buffler PA, Selvin S, *et al*. Daycare attendance and risk of childhood acute lymphoblastic leukamia. *Br J Cancer* 2002, **86**, 1419–1424.
8. Holt PG. Potential role of environmental factors in the etiology and pathogenesis of atopy: a working model. *Environ Health Perspect* 1999, **107**(Suppl 3), 485–487.
9. Kölmel KF, Pfahlberg A, Mastrangelo G, *et al*. Infections and melanoma risk: results of a multicentre EORTC case-control-study. *Melanoma Res* 1999, **9**, 511–519.
10. Pfahlberg A, Kölmel KF, Grange JM, *et al*. Inverse association between melanoma and previous vaccinations against tuberculosis and smallpox: results of the FEBIM study. *J Invest Dermatol* 2002, **119**, 570–575.
11. Krone B, Kölmel KF, Grange JM, *et al*. Impact of vaccinations and infectious diseases on the risk of melanoma – evaluation of an EORTC case-control-study. *Eur J Cancer* 2003, **39**, 2372–2378.
12. Kölmel KF, Grange JM, Krone B, et al. Prior immunisation of patients with malignant melanoma with vaccinia or BCG is associated with better survival. An European Organization for Research and Treatment of Cancer cohort study on 542 patients. *Eur J Cancer*, this issue (doi:10.1016/j.ejca.2004.09.023).
13. Leib-Mösch C, Seifarth W, Schön U. Influence of human endogenous retroviruses on cellular gene expression. In Sverdlow ED, ed. *Retroviruses and primate genome evolution*. Georgetown, TX, Eurekah.com, 2004.
14. Schiavetti F, Thonnard J, Colau D, Boon T, Coulie PG. A human endogenous retroviral sequence encoding an antigen recognized on melanoma by cytolytic T lymphocytes. *Cancer Res* 2002, **62**, 5510–5516.
15. Brehm MA, Pinto AK, Daniels KA, Schneck JP, Welsh RM, Selin LK. T cell immunodominance and maintenance of memory regulated by unexpectedly cross-reactive pathogens. *Nature Immunol* 2002, **3**, 627–634.
16. Koonin EV, Makarova KS, Yuri I, *et al*. In Syvanen M, Cado CI, eds. *Horizontal gene transfer*. 2nd ed. San Diego, USA, Academic Press, 2001. pp. 277–304.
17. Muster T, Waltenberger A, Grassauer A, *et al*. An endogenous retrovirus derived from human melanoma cells. *Cancer Res* 2003, **63**, 8735–8741.
18. Mayer J, Meese E, Mueller-Lantzsch N. Chromosomal assignment of human endogenous retrovirus K (HERV-K) env open reading frames. *Cytogen Cell Genet* 1997, **79**, 157–161.
19. De Parseval N, Lazar V, Casella JF, Benit L, Heidmann T. Survey of human genes of retroviral origin: Identification and transcriptome of the genes with coding capacity for complete envelope proteins. *J Virol* 2003, **77**, 10414–10422.
20. Cohen IR, Young DB. Autoimmunity, microbial immunity and the immunological homunculus. *Immunol Today* 1991, **12**, 105–110.
21. Ritter K, Härtl R, Bandlow G, Thomssen R. Cytostatic effect of gangliosides present in the membrane of macrophages. *Cell Immunol* 1986, **97**, 248–256.
22. Schaade L, Kleines M, Walter R, Thomssen R, Ritter K. A membrane-located glycosphingolipid of monocyte/granulocyte lineage cells induces growth arrest and triggers the lytic cylce in Epstein-Barr virus genome positive Burkitt-lymphoma lines. *Med Microbiol Immunol* 1999, **188**, 23–29.
23. Nojiri H, Kitagawa S, Nakamura M, Kirito K, Enomoto Y, Saito M. Neolacto-series gangliosides induce granulocytic differentiation of human promyelotic leukemia cell line HL-60. *J Biol Chem* 1988, **263**, 7443–7446.
24. Schaade L, Kleines M, Krone B, Hausding M, Walter R, Ritter K. Enhanced transcription of the S-adenosylhomocysteine hydrolase gene precedes Epstein-Barr virus lytic gene activation in ganglioside stimulated lymphoma cells. *Med Microbiol Immunol* 2000, **189**, 13–18.
25. Strausberg RL, Feingold EA, Grouse LH, *et al*. Generation and initial analysis of more than 15,000 full-length human and mouse cDNA sequences. *Proc Natl Acad Sci USA* 2002, **99**, 16899–16903.
26. Greer P. Closing in on the biological functions of Fps/Fes and Fer. *Nat Rev Mol Cell Biol* 2002, **3**, 278–289.
27. Okada M, Ogasawara H, Kaneko H, *et al*. Role of DNA methylation in transcription of human endogenous retrovirus in the pathogenesis of systemic lupus erythematosus. *J Rheumatol* 2002, **29**, 1678–1682.
28. Florl AR, Lower R, Schmitz-Drager BJ, Schulz WA. DNA methylation and expression of LINE-1 and HERV-K provirus sequences in urothelial and renal cell carcinomas. *Br J Cancer* 1999, **80**, 1312–1321.

29. Sugimoto J, Matsuura N, Kinjo Y, Tagasu N, Oda T, Jinne Y. Transcriptionally active HERV-K genes: identification, isolation, and chromosomal mapping. *Genomics* 2001, **72**, 137–144.
30. Bolot G, David MJ, Taki T, *et al*. Analysis of glycosphingolipids of human head and neck carcinomas with comparison of normal tissue. *Biochem Mol Biol Int* 1998, **46**, 125–135.
31. Kong Y, Li R, Ladisch S. Natural forms of shed tumor gangliosides. *Biochem Biophys Acta* 1998, **1394**, 43–56.
32. Kanda N, Nakai K, Watanabe S. Gangliosides GD1b, GT1b, and GQ1b suppress the growth of human melanoma by inhibiting interleukin-8 production: the inhibition of adenylate cyclase. *J Invest Dermatol* 2001, **117**, 284–293.
33. Hakomori S, Yamamura S, Handa AK. Signal transduction through glycol/(sphingo)lipids. Introduction and recent studies on glycol/(sphingo)lipid-enriched microdomains. *Ann NY Acad Sci* 1998, **845**, 1–10.
34. Mangeney M, de Parseval N, Thomas G, Heidmann T. The full-length envelope of an HERV-H human endogenous retrovirus has immunosuppressive properties. *J Gen Virol* 2001, **82**, 2515–2518.
35. Moore M, Lawrence N, Nisbet NW. Inhibition of transplanted sarcomas mediated by BCG in rats with a defined immunological deficit. *Biomedicine* 1976, **24**, 26–31.
36. Hoon DS, Hayashi Y, Morisaki T, Foshag LJ, Morton DL. Interleukin-4 plus tumor necrosis factor alpha augments the antigenicity of melanoma cells. *Cancer Immunol Immunother* 1993, **37**, 378–384.
37. Jun JE, Goodnow CC. Scaffolding of antigen receptors for immunogenic versus tolerogenic signalling. *Nature Immunol* 2003, **4**, 1057–1064.
38. Weiss A, Schlessinger J. Switching signals on or off by receptor dimerization. *Cell* 1998, **94**, 277–280.
39. Nojiri H, Stroud M, Hakomori S. A specific type of ganglioside as a modulator of insulin-dependent cell growth and insulin receptor tyrosine kinase activity. *J Biol Chem* 1991, **266**, 4531–4537.
40. Merante F, Altamentova SM, Mickle DA, *et al*. The characterization and purification of a human transcription factor modulating the glutathione peroxidase gene in response to oxygen tension. *Mol Cell Biochem* 2002, **229**, 73–83.
41. Horsley V, Pavlath GK. NFAT: ubiquitous regulator of cell differentiation and adaptation. *J Cell Biol* 2002, **156**, 771–774.
42. Weinlich G, Bitterlich W, Mayr V, Fritsch PO, Zelger B. Metallothionein-overexpression as a prognostic factor for progression and survival in melanoma. A prospective study on 520 patients. *Br J Dermatol* 2003, **149**, 535–541.
43. Meyskens FL, Farmer P, Fruehauf JP. Redox regulation in human melanocytes and melanoma. *Pigment Cell Res* 2001, **14**, 148–154.
44. Salvucci O, Carsana M, Bersani I, Tragni G, Anichini A. Antiapoptotic role of endogenous nitric oxide in human melanoma cells. *Cancer Res* 2001, **61**, 318–326.
45. Palozza P, Serini S, Torsello A, *et al*. Mechanism of activation of caspase cascade during beta-carotene-induced apoptosis in human tuomr cells. *Nutr Cancer* 2003, **47**, 76–87.
46. Fukuzawa K, Kogure K, Morita M, Hama S, Manabe S, Tokumura A. Enhancement of nitric oxide and superoxide generations by alpha-tocopheryl succinate and its apoptotic and anticancer effects. *Biochemistry (Mosc)* 2004, **69**, 50–57.
47. Lopez-Saez JB, Senra-Varela A, Pousa-Estevez L. Selenium in breast cancer. *Oncology* 2003, **64**, 227–231.
48. Postovsky S, Arush MW, Diamond E, Elhasid R, Shoshani G, Shamir R. The prevalence of low selenium levels in newly diagnosed pediatric cancer patients. *Pediatr Hematol Oncol* 2003, **20**, 273–280.
49. Oldfield JE. Selenium World Atlas, Updated edition. Grimbergen (Belgium), Selenium–Tellurium Development Association, 2002.
50. Kardinaal AF, Kok FJ, Kohlmeier L, *et al*. Association between toenail selenium and risk of acute myocardial infarction in European men. *Am J Epidemiol* 1997, **145**, 373–379.
51. World Health Organization. Global tuberculosis control: surveillance, planning, financing. WHO Report 2002. Geneva, World Health Organization, 2002 (Publication WHO/CDS/TB/2002.295).
52. Stanford JL, Stanford CA, Grange JM. Environmental echoes. *Science Prog* 2001, **84**(Part 2), 105–124.
53. Rook GA, Stanford JL. Give us this day our daily germs. *Immunol Today* 1998, **19**, 113–116.
54. Anonymous. Original antigenic sin. *N Engl J Med* 1958, **258**, 1016–1017.
55. Kaempfer R, Gerez L, Farbstein H, *et al*. Prediction of response to treatment in superficial bladder carcinoma through pattern of interleukin-2 gene expression. *J Clin Oncol* 1996, **14**, 1778–1786.
56. Stanford JL, Stanford CA, Baban B, Grange JM. Therapeutic vaccination for cancer: the potential value of mycobacterial products. *Int J Pharmaceut Med* 1999, **12**, 191–195.
57. Maraveyas A, Baban B, Kennard D, *et al*. Possible improved survival of patients with stage IV AJCC melanoma receiving SRL 172 immunotherapy: correlation with induction of increased levels of intracellular interleukin-2 in peripheral blood lymphocytes. *Ann Oncol* 1999, **10**, 817–824.
58. Grange JM, Stanford JL, Stanford CA, Kölmel KF. Prospects for vaccination strategies to reduce the risk of leukaemia and melanoma. *J Roy Soc Med* 2003, **96**, 389–392.
59. Rook G, Baker R, Walker B, *et al*. Local regulation of glucocorticoid activity in sites of inflammation. Insights from the study of tuberculosis. *Ann NY Acad Sci* 2000, **917**, 913–922.
60. Andersson AC, Svensson AC, Rolny C, Andersson G, Larsson E. Expression of human endogenous retrovirus ERV3 (HERV-R) mRNA in normal and neoplastic tissues. *Int J Oncol* 1998, **12**, 309–313.
61. Garly ML, Martins CL, Bale C, *et al*. BCG scar and positive tuberculin reaction associated with reduced child mortality in West Africa. A non-specific beneficial effect of BCG? *Vaccine* 2003, **21**, 2782–2790.