[Frontiers in Bioscience S4, 1393-1401, June 1, 2012]

Peptide cross-reactivity: the original sin of vaccines

Darja Kanduc[1]

[1]*Department of Biochemistry and Molecular Biology, University of Bari, Bari Italy*

TABLE OF CONTENTS

1. ABSTRACT

Recent studies have demonstrated that an extensive peptide identity platform characterizes entities spanning the entire evolutionary arc from viruses to humans. These studies also established existence of an immune cross-reactivity among viruses and bacteria, as well as between microbial organisms and humans. This peptide commonality presents obstacles to diagnostics, burdens therapeutic vaccinology with harmful collateral effects, and can result in autoimmune diseases. The present study 1) recapitulates the significance of cross-reactivity from the molecular mimicry hypothesis to the phenomenon of microbial immunoevasion; 2) analyzes the implications of cross-reactivity for the self-nonself discrimination issue; 3) highlights the negative role exerted by cross-reactions in translating immunology to effective vaccines; 4) outlines the vicious circle connecting peptide commonality, microbial immune escape, adjuvanted vaccines and autoimmune cross-reactions; and 5) conclusively indicates sequence uniqueness as a basic criterion for designing effective vaccines exempt from autoimmune cross-reactions.

2. INTRODUCTION: THE "VACCINE PROBLEM"

Immunology has accumulated a tremendous amount of knowledge on the components of the immune system and the structural-functional correlates underlying their function. However, the advantages expected from this massive knowledge have not concretized. Citing verbatim Coutinho: "*...we continue to treat allergy as we did before IgE was known, we have no specific therapy for autoimmune diseases, we are unable to tolerize the recipient of an organ to the tissues of the donor, and we seem incompetent to derive vaccines to protect the larger part of the world population from parasite infections*" (1). Of note, Coutinho's observations date to more than 20 years ago. At that time, they invoked the importance of investing more in the description of genes, molecules and cells in order to move toward translational medicine. It was also argued that a shift from molecular and cellular immunology to systemic immunology was necessary to address those immunological issues that remained unresolved.

In 2011, we continue to rely mainly on immunosuppressors for treating autoimmune diseases,

register data on failed efforts to develop anti-tumor vaccines, and still face limitations in designing effective novel vaccines for infectious diseases. In addition, unfortunately we witness today an ever-rising aversion to vaccination protocols because of the numerous putative and/or real adverse events associated with the vaccine formulations currently in use. It seems science has not been able to explain, overcome, and solve vaccination fears dating back more than two hundred years ago (2).

It is suggested that the data obtained from common experimental mouse models may be inadequate to understand and treat human immunological diseases (3). Moreover, it is said that the experimental conditions used in mouse systems (such as the amounts and routes of administration of the antigen and adjuvant) cannot be extrapolated to humans (4). Here, we suggest that peptide cross-reactivity is a basic factor affecting the efficacy of vaccines and the future of human immunology. In particular, we review the extent and role of peptide cross-reactivity between microbial and human proteins, and suggest that the difficulties in designing "the right vaccines" against infectious agents are possibly intrinsic to the human-affine nature of the microbial proteomes.

3. ANTIBODIES AND ANTIGENS: CROSS-REACTIVITY BEFORE MONOCLONAL ANTIBODIES

From the beginning of the immunological era, researchers recognized that cross-reactivity was a major obstacle to the development of immunoassay systems, as described in the following brief historical snapshot: 1) In 1939, Eagle and Hogan (5) observed that an alcoholic extract of normal mammalian tissue can be used as 'antigen' instead of the aqueous extract of syphilitic liver, thus rendering the Wassermann reaction a puzzling anomaly among serodiagnostic tests. 2) Antibodies directed against human follicle-stimulating hormone (FSH) failed to distinguish the four human glycoprotein hormones: FSH, luteinizing hormone, chorionic gonadotropin, and thyrotropin (6). 3) Even immunoassay of aldosterone, a small molecular weight compound, was subjected to the interference such as that exerted by hydroxylated metabolites of spirolactones (7). 4) Extensive cross-reactivity is found among microbial organisms (8). 5) The cross-reactivity issue is further complicated by inter-species reactions. For example, rabbit serum antibody raised against beef heart cytochrome oxidase quantitatively reacted with rat liver cytochrome oxidase (9).

Pari passu, research highlighted the intersection of cross-reactivity and immunopathology. The ability of the antihypertensive drug hydralazine to induce a syndrome similar to spontaneous systemic lupus erythematosus (SLE) in humans was related to the fact that anti-hydralazine serum consisted of antibodies to hydralazine and progressively increasing amounts of antibodies to both single-stranded and native DNA. In essence, antibodies to nuclear antigens were related to SLE, thus indicating that a cross-reactive immune response to drugs might be important in human autoimmunity (10). Likewise, cross-reactivity between the polysaccharide of group A streptococcus and the glycoprotein of heart valves was identified as a primum movens in the pathogenesis of rheumatic heart disease (11).

On the whole, evidence that immune responses are characterized by cross-reactivity accumulated gradually. At that time, such cross-reactivity was adduced to indicate that immune sera contain mixtures of antibody molecules with a large variety of specificities.

4. ANTIBODIES AND ANTIGENS: CROSS-REACTIVITY AFTER MONOCLONAL ANTIBODIES

The unresolvable complexity of cross-reactivity emerged as a relevant phenomenon with the discovery of monoclonal antibodies (MAbs), i.e., antibodies that were expected to have an exquisitely unique specificity by being produced by identical lymphocytes derived from a unique parent cell (12). This soon seemed not to be the case. In 1981, Dulbecco *et al.* (13) demonstrated, using a monoclonal antibody, cross-reactivity between Thy-1 and a component of intermediate filaments. From then on, there has been a continuum of experimental data demonstrating Mab polyspecific reactivity. Cited below are a few random examples: 1) In competitive radioimmunoassays, cardiolipin, phosphatidic acid, and phosphatidyl glycerol blocked the binding of MAbs reactive with polynucleotides (14). 2) A monoclonal anti-DNA autoantibody (EM85) was reported to bind to proteins on the surface of Raji cells (15). 3) Polyspecific reactivity of a MAb (RTE-23) with cytoskeletal proteins (vimentin, keratin, actin), single-stranded DNA, specific synthetic polynucleotides, and cardiolipin (16) was reported. 4) MAbs sharing specificities with mouse natural MAbs were described and characterized in Avrameas' laboratory (17). Furthermore, multiple organ-reactivity of MAbs to mouse erythrocytes was described (18). 5) Anti-tetanus toxoid MAbs were found to cross-react with diphtheria toxoid, cardiolipin, and DNA, thus indicating that cross-reactive epitopes occur on routinely used toxoid vaccines and self antigens (19). 6) To add complexity, two unrelated MAbs, HyHEL-10 and F9.13.7, recognize the same epitope of hen egg-white lysozyme (20).

Currently, polyspecific reactivity of a single molecular species of antibody continues to be under scientific scrutiny because it represents a major obstacle to the development of immunotherapy effective against devastating infections such as HIV. Indeed, anti-HIV immunotherapy is hampered by the concern of inducing collateral autoimmune phenomena through the induction of humoral/cellular responses that could be cross-reacting with the host proteome. In fact, human monoclonal IgM antibodies to the synthetic peptide MN-24 corresponding to 303-325 residues of gp120 HIV have been found to be reactive not only with different short peptides, but with some macromolecular antigens as well (21). Likewise, the two most broadly reactive HIV-1 envelope gp41 human MAbs, 2F5 and 4E10, are polyspecific autoantibodies reactive with the phospholipid cardiolipin. MAb 2F5 also reacts with histones and the centromere B autoantigen, whereas MAb 4E10 reacts with the systemic SLE autoantigen SS-A/Ro. Both 2F5 and 4E10 also react with

HEp-2 human epithelial cells in a diffuse cytoplasmic and nuclear pattern, further revealing polyspecific autoreactivity of both antibodies (22). Taken together, the above mentioned data challenge the concept of antibody exquisite specificity and support the theory of a general polyspecific potential of MAbs (23).

5. CROSS-REACTIVITY AND THE MOLECULAR MIMICRY HYPOTHESIS

The term "molecular mimicry" was borrowed from the naturalistic sciences debate occurring in the 1930's (24) . In that frame, mimicry accounted for the following sentence: "*an insect escapes being eaten because it reminds the enemy of an object which he is not accustomed to eat*" (24). Then, in the 1960's, Raymond Damian was the first to translate the naturalistic mimicry concept to the molecular immunological context. In studying parasites, he assumed that parasite antigenic determinants resembling those of the host do not elicit immune reactions because of their being similar to the host (25). Therefore, molecular mimicry initially indicated a camouflage phenomenon to avoid recognition. Historically, this is the first nexus between molecular mimicry and microbial escape from immune surveillance, a phenomenon that remains an object of ongoing scientific discussion (26-30).

However, Damian's molecular mimicry concept took a turn for the contrary. Almost contemporaneously, in 1962 Rowley and Jenkin (31) suggested that infections might lead to autoimmune disease as a result of cross-reactive antibodies or T cells, thus starting a research era aimed at demonstrating that sharing of amino acid sequences or structures between microbes and humans was at the root of autoimmune diseases (32-35). In this new interpretation of the molecular mimicry concept, the immune system "sees", "recognizes" and "attacks" the pathogen sequences/structures. In so acting, the immune system does not pay attention to the fact that the same sequences/structures may be present in the host. Therefore, in reacting against the pathogen, the immune system cross-reacts with identical or similar host sequences/structures, thus breaking the host immunotolerance status and producing autoimmune responses, and, consequently, autoimmune diseases. However, following three decades of intensive research in the field, a causative link between molecular mimicry and human autoimmune diseases is still sub judice, and the molecular mimicry hypothesis has received no validation (36-39).

6. CROSS-REACTIVITY AND THE PHENOMENON OF MICROBIAL ESCAPE FROM IMMUNE SURVEILLANCE

During the last decade, Kanduc and others (40-46) described a massive peptide overlap between viral proteins and the human proteome. Only a fraction of sequences (less than 10% at the pentapeptide level) are unique to viruses and not found in the human proteome (42). In addition, the exploration of bacterial proteomes for peptides matching the human proteome revealed a sharing of millions of peptide sequences (47-51). In particular, analysis of 40 bacterial proteomes showed that not a single protein is exempt from bacterial motifs at the hexapeptide level (49). The peptide commonality between microbes and humans is illustrated in Figure 1, showing the ratio of human proteins containing bacterial heptapeptide(s) (99,7%) and those that do not (0.3 %). That is, Figure 1 illustrates the concept that only a tiny fraction of the approximately 30,000 proteins that form the human proteome is exempt from bacterial heptapeptide motifs.

The massive peptide overlap between viruses/bacteria and humans is of impact in the immunologic context and demonstrates the fragility of the molecular mimicry hypothesis. In practice, the data obtained by Kanduc and others (40-51) scientifically validate, at the molecular level, Damian's intuition (23) according to which the parasite antigenic determinants resembling those of the host would not elicit immune reactions because of their being similar to the host. The massive usage of the same peptide blocks in microbial and human proteomes inficiates any causal relationship(s) between sharing of microbial amino acid motifs/structures and autoimmune reactions in humans. The molecular mimicry hypothesis cannot explain autoimmune diseases since, if there were a link between sharing of viral/bacterial peptides and human autoimmune reactions, then the massive extent of viral/bacterial peptide overlap in the human proteome would cause the worldwide human population to suffer from autoimmunity with a 100% incidence. E contrario, it seems that the imponent microbial-versus-human peptide overlap offers a proteomic niche for microbes to conceal themselves, thus escaping specific immune recognition. According to Kanduc (52, 53), viruses and bacteria are "a portion" of our human self and are subject to the same tolerogenic mechanisms that characterize human antigens and tissues. This being the case, the self-nonself debate is a pure confrontation between proteomes, with the motifs uniquely owned by microbial entities as unique "nonself" targets of potential immune interactions (54).

Based on the massive peptide overlap between viruses/bacteria and humans, Kanduc (52) also affirms that only those amino acid sequences uniquely expressed in a proteome may have an immune potential. *De facto*, Kanduc (53-55) finds that almost all epitopes, independent of their being derived from cancer antigens, viral/bacterial proteins, autoantigens or allergens, are located in low-similarity antigen areas. As portrayed in Figure 2, immunological information is packed into rare peptide motifs (56).

Finally, as a coherent conclusion, Kanduc (52-54) draws a link between the bacterial/viral peptide overlap with the human proteome and immunopathology. In fact, groupings of 5-6 aa are minimal immune determinants in antigen–antibody recognition (57-59, 60 and pertinent references therein). In addition T-cell immune units appear to be contained in an epitopic space defined by 5–6 amino acid residues (61-64). In general, it is well known that regulation of immunomodulation can be exerted by pentapeptides (65-72) and, even, tetrapeptides (63, 68). Hence, vaccination protocols inducing an immune response against whole antigens from viruses/bacteria may also induce a vast array of autoimmune responses in the

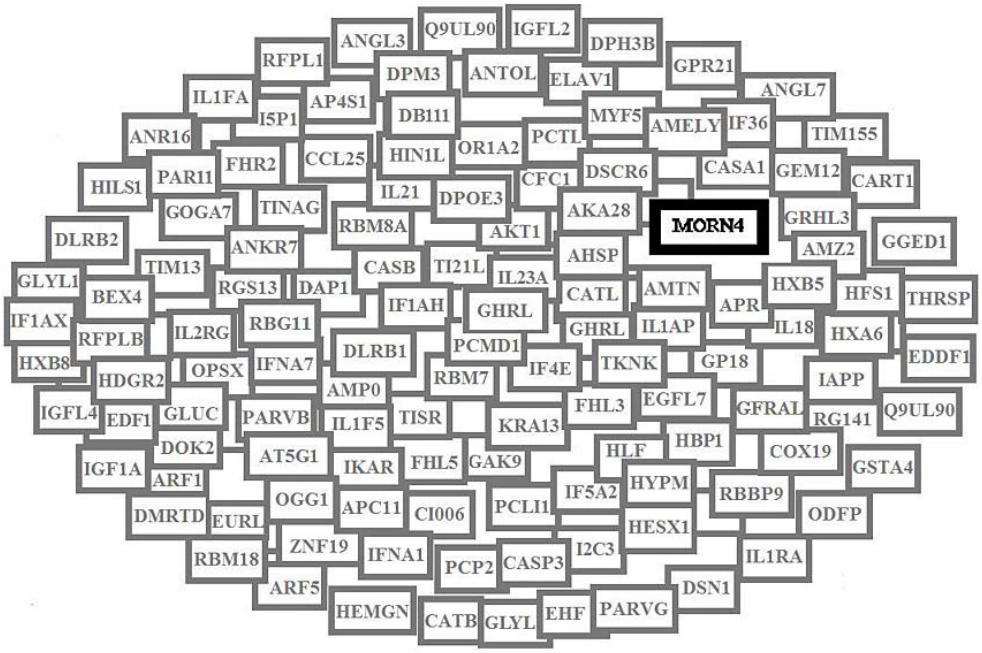

Figure 1. Bacterial peptides are pervasively distributed throughout the human proteins. A schematic representation of the peptide sharing between bacterial proteomes and the human proteome at the heptapeptide level. Human proteins reported as UniProt/Swiss entries. Human proteins containing bacterial heptapeptide(s) are boxed in gray. Human proteins with no bacterial heptapeptide(s) are boxed in black. Further data in Refs. 48, 49.

vaccinated host because of the widespread pentapeptide sharing between viral/bacterial proteins and the human proteome (43-46). As an example, a vaccine based on the Influenza A H5N1 polyprotein might well destroy the virus but, at the same time, may hit human neuronal antigens that contain viral peptide sequences (Figure 3) (46).

7. PEPTIDE COMMONALITY, IMMUNE ESCAPE, ADJUVANTED VACCINES AND AUTOIMMUNE CROSS-REACTIONS. A VICIOUS CIRCLE.

As reasoned above and elsewhere (41-46, 48-54), the existence of a widespread peptide commonality between microbes and humans might be a contributing factor in determining microbial immune escape. Consequently, it may also explain the fact that, in general, active vaccines based on antigens from infectious agents produce a weak (or no) immune response. That is, because of immunotolerogenic mechanisms towards repeatedly shared peptide motifs, the human immune system may fail to react against the infectious antigens present in the vaccines. In general, active vaccines are weakly immunogenic. This scarce vaccine immunogenicity has made the use of adjuvants necessary (73-76). Adjuvants include inorganic compounds (heavy metals, aluminium hydroxide, aluminium phosphate, and calcium phosphate) (77,78), glycosphingolipid and oil emulsions (79), and products from bacteria (e.g., lipopolysaccharides and lectins) (80). In practice, adjuvants are highly heterogeneous chemical compounds, with the common property of stimulating immune responses.

On the other hand, adjuvant-induced hyperactivation of the immune system may alter/silence the still ill-defined tolerigenic mechanisms that keep the immune system under control and lead to the avoidance of harmful auto-attacks. Hence, as a *logica consecutio*, following adjuvanted vaccination, aspecific reactions may occur against host molecules/ structures because of the massive peptide matching between microbes and humans, thus starting autoimmune processes. The type of autoimmune phenomenon and disease that is eventually established will depend on the molecules and organs attacked. For example, attacks against myelin and myelin-related structures/enzymes may evoke demyelinating diseases, whereas immune reactions against proteins and antigens involved in behavior and/or cognition (neurobeachin, adenosine deaminase, neuroligin, reelin, etc) may cause autism and behavior disorders. In other words, the peptidome platform shared by the antigens present in vaccines and the human host plays a fundamental role in dictating which autoimmune disease occurs following adjuvant-induced immunogenicity.

In synthesis: peptide commonality, microbial immunoevasion, low efficacy of vaccines, adjuvant usage, and autoimmune cross-reactions may constitute a vicious circle leading to autoimmunity following vaccination.

8. THE FUTURE OF VACCINES: THE CONCEPT OF SEQUENCE UNIQUENESS

In the late 1700's, Edward Jenner introduced vaccination as a tool against infectious diseases. Infecting oneself with the infectious agent to avoid the disease

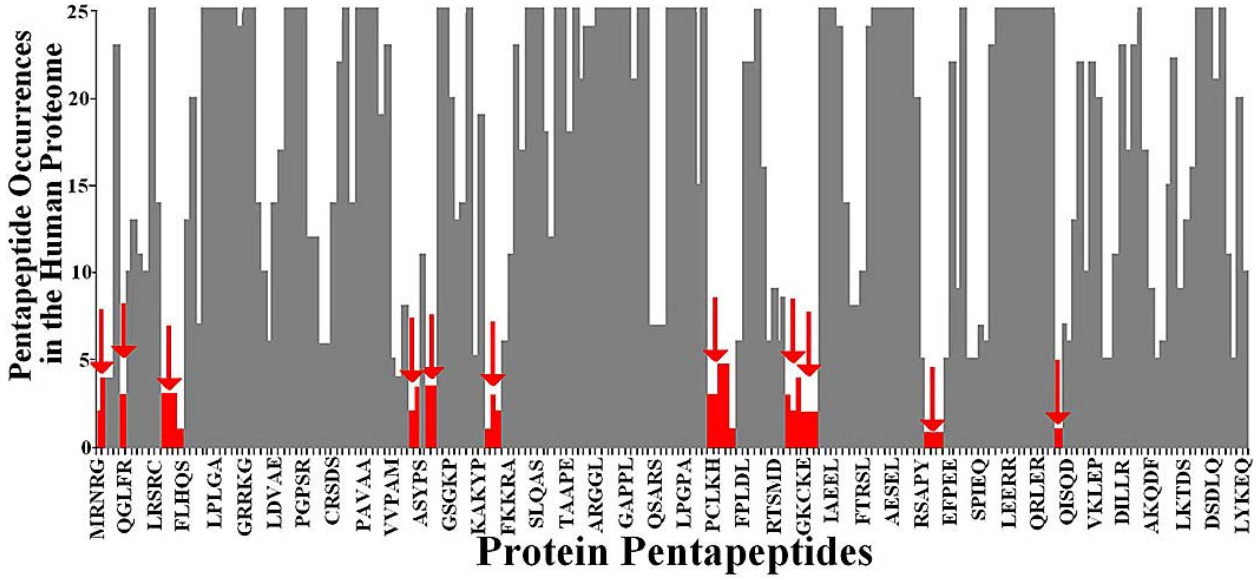

Figure 2. The concept: rare peptide sequences are potential epitopes. Arrows indicate potential epitopic sequences allocated in low-similarity areas (in red) along the primary sequence of a protein.

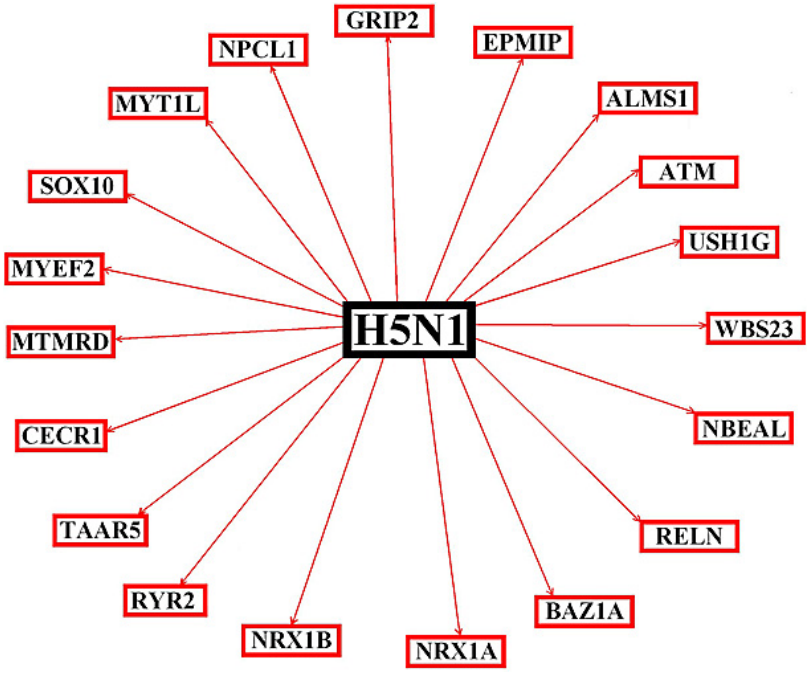

Figure 3. Aiming at influenza A H5N1 virus, hitting the brain. A schematic representation of human neuronal antigens sharing peptide motifs with influenza A H5N1 polyprotein. Human neuronal antigens are boxed in red and indicated by UniProt/Swiss entries. Further data in Ref. (46). From left: MTMRD) Myotubularin-related protein 13. Expressed in spinal cord; MYEF2) Myelin expression factor 2; SOX10) Transcription factor SOX-10; MYT1L) Myelin transcription factor 1-like protein; NPCL1) Niemann–Pick C1-like protein 1; GRIP2) Glutamate receptor-interacting protein 2; EPMIP) EPM2A interacting protein 1 or Laforin-interacting protein; ALMS1) Alstrom syndrome protein 1; ATM) Ataxia–telangiectasia mutated protein; USH1G) Usher syndrome type-1G protein; WBS23) Williams–Beuren syndrome chromosomal region 23 protein; NBEAL) NBEA L1 protein or amyotrophic lateral sclerosis 2 chromosomal region candidate gene 17 protein; RELN) Reelin. Plays a role in layering of neurons in the cerebral cortex and cerebellum; BAZ1A) Williams syndrome transcription factor related chromatin-remodelling factor 180; NRX1A) Neurexin I-alpha; NRX1B) Neurexin-1-beta; RYR2) Ryanodine receptor 2; TAAR5) Trace amine-associated receptor 5; CECR1) Adenosine deaminase CECR1 also called Cat eye syndrome critical region protein 1.

became popular around the world. However, the practice of vaccination was soon under attack (81). Prominent scientists and philosophers such as Alfred Russell Wallace, Immanuel Kant, and Herbert Spencer, were involved in the critical debate against vaccination (82, 83). Then as today, the injuriousness of vaccination was under accuse. One statement was that phthisis, cancer, and madness are likely to be the products of vaccination since they increased in frequency after vaccination was introduced (84). Today, increased autism, childhood leukemia, and cardiac failure are listed among the potential consequences of vaccination.

However, the anti-vaccine debate was and still is sterile (2, 81). No proof of a direct relationship between vaccination and adverse events has been brought to the attention of the scientific and medical communities, and the presumed vaccination-associated damages have been proposed and analyzed only in epidemiological, and often incomplete, terms.

In this context, the present study represents the first clear-cut meta-analysis of a molecular platform able to rationalize the potential cause-effect link between vaccination and subsequent adverse events. The data discussed here delineate peptide commonality between microbes and humans as the Wallacian “harmonia naturae” protected by immunotolerance and broken by adjuvanted vaccines.

Importantly, the data discussed here also indicate new avenues in vaccinology. Together with a number of recent reports from this lab (85-97), the present study demonstrates that only peptide motifs absent or scarcely represented in the host proteome appear to have the potential to evoke a safe, targeted, and effective immune response. Vaccine preparations based on peptide fragments unique to the infectious agent are not only expected to specifically hit the agent without harmful cross-reactions with host proteins, but also may bypass escape mechanisms because of their being practically unknown to the immune system, thus eliminating a main obstacle to an effective immune recognition and destruction of the infectious agent. A successful future looms for vaccines with unique peptide sequences.

9. REFERENCES

1. Coutinho A: Beyond clonal selection and network. *Immunol Rev* 110, 63-87 (1989)

2. M Fichman, JE Keelan: Resister's logic: the anti-vaccination arguments of Alfred Russel Wallace and their role in the debates over compulsory vaccination in England, 1870-1907. *Stud Hist Philos Biol Biomed Sci* 38, 585-607 (2007)

3. MM Davis: A prescription for human immunology. *Immunity* 29, 835-838 (2008)

4. RN Germain: Vaccines and the future of human immunology. *Immunity* 33, 441-450 (2010)

5. H Eagle, RB Hogan: On the presence in syphilitic serum of antibodies to spirochetes, their relation to so called Wassermann reagin, and their significance for the serodiagnosis of syphilis. *J Exp Med* 71, 215-230 (1940)

6. S Schlaff, SW Rosen, J Roth: Antibody to human follicle-stimulating hormone: cross-reactivity with three other hormones. *J Clin Invest* 47, 1722-1729 (1968)

7. W Sadee, AM Finn, P Schmiedek, A Baethmann: Aldosterone plasma radioimmunoassay interference by a spirolactone metabolite. *Steroids* 25, 301-311 (1975)

8. SL Keasey, KE Schmid, MS Lee, J Meegan, P Tomas, M Minto, AP Tikhonov, B Schweitzer, RG Ulrich: Extensive antibody cross-reactivity among infectious gram-negative bacteria revealed by proteome microarray analysis. *Mol Cell Proteomics* 8, 924-935 (2009)

9. WB Elliott, JP Holbrook, R Penniall: Studies on a cytochrome oxidase antibody. III. Cross reactivity. *Biochim Biophys Acta* 251, 277-280 (1971)

10. Y Yamauchi, A Litwin, L Adams, H Zimmer, EV Hess: Induction of antibodies to nuclear antigens in rabbits by immunization with hydralazine-human serum albumin conjugates. *J Clin Invest* 56, 958-969 (1975)

11. S Kawakita, H Iwamoto: Studies on the pathogenesis of rheumatic heart disease. An immunological relationship between the polysaccharide of group A streptococcus and the glycoprotein of heart valve. *Jpn Circ J* 39, 439-446 (1975)

12. G Kohler, C Milstein: Continuous cultures of fused cells secreting antibody of predefined specificity. *Nature* 256, 495-497 (1975)

13. R Dulbecco, M Unger, M Bologna, H Battifora, P Syka, S Okada: Cross-reactivity between Thy-1 and a component of intermediate filaments demonstrated using a monoclonal antibody. *Nature* 292, 772-774 (1981)

14. EM Lafer, J Rauch, C Andrzejewski Jr, D Mudd, B Furie, B Furie, RS Schwartz: Polyspecific monoclonal lupus autoantibodies reactive with both polynucleotides and phospholipids. *J Exp Med* 153, 897-909 (1981)

15. E Muso, L Jacob: A polyspecific monoclonal anti-DNA autoantibody also binds to cell-surface protein(s). *Clin Immunol Immunopathol* 42, 370-374 (1987)

16. AJ Laster, DS Pisetsky, BF Haynes: Polyspecific reactivity of a murine monoclonal antibody that binds to nuclear matrix-associated, chromatin-bound autoantigens. *Clin Immunol Immunopathol* 44, 187-205 (1987)

17. T Ternynck, S Avrameas: Murine natural monoclonal autoantibodies: a study of their polyspecificities and their affinities. *Immunol Rev* 94, 99-112 (1986)

18. C Garzelli, Basolo F, Puglisi C, Pacciardi A: Multiple organ-reactivity of monoclonal autoantibodies to mouse erythrocytes. *Experientia* 43, 912-914 (1987)

19. M Sutjita, A Hohmann, R Comacchio, J Bradley: Polyspecific human and murine antibodies to diphtheria and tetanus toxoids and phospholipids. *Clin Exp Immunol* 73, 191-197 (1988)

20. J Pons, JR Stratton, JF Kirsch: How do two unrelated antibodies, HyHEL-10 and F9.13.7, recognize the same epitope of hen egg-white lysozyme? *Protein Sci* 11, 2308-2315 (2002)

21. E Sidorova: Human monoclonal antibodies to MN-24 peptide of gp 120 HIV-1. *Hum Antibodies* 9, 107-110 (1999)

22. BF Haynes, J Fleming, EW St Clair, H Katinger, G Stiegler, R Kunert, J Robinson, RM Scearce, K Plonk, HF Staats, TL Ortel, HX Liao, SM Alam: Cardiolipin polyspecific autoreactivity in two broadly neutralizing HIV-1 antibodies. *Science* 308, 1906-1908 (2005)

23. L Otte, T Knaute, J Schneider-Mergener, A Kramer: Molecular basis for the binding polyspecificity of an anti-cholera toxin peptide 3 monoclonal antibody. *J Mol Recognit* 19, 49-59 (2006)

24. GD Carpenter: Mimicry, as viewed by Professor Shull. *Science* 85, 356-359 (1937)

25. RT Damian: Molecular mimicry: antigen sharing by parasite and host and its consequences. *Am Naturalist* 98, 129–149 (1964)

26. JT Weinfurter, GE May, T Soma, AJ Hessell, Leon EJ, CE Macnair, SM Piaskowski, K Weisgrau, J Furlott, NJ Maness, J Reed, NA Wilson, EG Rakasz, DR Burton, TC Friedrich: Macaque long-term nonprogressors resist superinfection with multiple CD8+ T cell escape variants of simian immunodeficiency virus. *J Virol* 85, 530-541 (2011)

27. S Yokota, T Okabayashi, N Fujii: The battle between virus and host: modulation of Toll-like receptor signaling pathways by virus infection. *Mediators Inflamm* 184328 (2010)

28. M Ota, BH Duong, A Torkamani, CM Doyle, AL Gavin, T Ota, D Nemazee: Regulation of the B cell receptor repertoire and self-reactivity by BAFF. *J Immunol* 185, 4128-4136 (2010)

29. IE Frohner, C Bourgeois, K Yatsyk, O Majer, K Kuchler: *Candida albicans* cell surface superoxide dismutases degrade host-derived reactive oxygen species to escape innate immune surveillance. *Mol Microbiol* 71, 240-252 (2009)

30. JD Lambris, D Ricklin, BV Geisbrecht: Complement evasion by human pathogens. *Nat Rev Microbiol* 6, 132-142 (2008)

31. D Rowley, CR Jenkin: Antigenic cross-reaction between host and parasite as a possible cause of pathogenicity. *Nature* 193, 151-154 (1962)

32. RF Shapiro, KB Wiesner, BL Bryan, PD Utsinger, D Resnick, JJ Castles: HLA-B27 and modified bone formation. *Lancet* 307, 230-231 (1976)

33. A Ebringer: Ankylosing spondylitis, immune-response-genes and molecular mimicry. *Lancet* 313, 1186 (1979)

34. RS Fujinami, MB Oldstone, Z Wroblewska, ME Frankel, H Koprowski: Molecular mimicry in virus infection: crossreaction of measles virus phosphoprotein or of herpes simplex virus protein with human intermediate filaments. *Proc Natl Acad Sci USA*; 80, 2346-2350 (1983)

35. LJ Albert, RD Inman: Molecular mimicry and autoimmunity. *N Engl J Med* 341, 2068-2074 (1999)

36. C Benoist, D Mathis: Autoimmunity provoked by infection: how good is the case for T cell epitope mimicry? *Nat Immunol* 2, 797-801 (2001)

37. CW Ang, BC Jacobs, JD Laman: The Guillain-Barré syndrome: a true case of molecular mimicry. *Trends Immunol* 25, 61-66 (2004)

38. JE Libbey, LL McCoy, RS Fujinami: Molecular mimicry in multiple sclerosis. *Int Rev Neurobiol* 79, 127-147 (2007)

39. S Acharya, S Shukla, SN Mahajan, SK Diwan: Molecular mimicry in human diseases: phenomena or epiphenomena? *J Assoc Physicians India* 58, 163-168 (2010)

40. A Kusalik, M Bickis, C Lewis, Y Li, G Lucchese, FM Marincola, D Kanduc: Widespread and ample peptide overlapping between HCV and *Homo sapiens* proteomes. *Peptides* 28, 1260-1267 (2007)

41. D Kanduc, A Stufano, G Lucchese, A Kusalik: Massive peptide sharing between viral and human proteomes. *Peptides* 29, 1755-1766 (2008)

42. D Kanduc: Quantifying the possible cross-reactivity risk of an HPV16 vaccine. *J Exp Ther Oncol* 8, 65-76 (2009)

43. D Kanduc: Penta- and hexapeptide sharing between HPV16 and *Homo sapiens* proteomes. *Int J Med Med Sci* 1, 387 (2009)

44. D Kanduc, A Lucchese, A Mittelman: Non-redundant peptidomes from DAPs: towards "the vaccine"? *Autoimmun Rev* 6, 290-294 (2007)

45. R Ricco, D Kanduc: Hepatitis B virus and *Homo sapiens* proteome-wide analysis: A profusion of viral peptide overlaps in neuron-specific human proteins. *Biologics* 4, 75-81 (2010)

46. D Kanduc: Describing the hexapeptide identity platform between the influenza A H5N1 and *Homo sapiens* proteomes. *Biologics* 4, 245-261 (2010)

47. G Lucchese, A Stufano, D Kanduc: Proposing low-similarity peptide vaccines against *Mycobacterium tuberculosis*. *J Biomed Biotechnol* 832341 (2010)

48. B Trost, A Kusalik, G Lucchese, G Kanduc: Bacterial peptides are intensively present throughout the human proteome. *Self Nonself* 1, 71-74 (2010)

49. B Trost, G Lucchese, A Stufano, M Bickis, A Kusalik, D Kanduc D: No human protein is exempt from bacterial motifs, not even one. *Self Nonself* 1, 328-334 (2010)

50. SL Bavaro, D Kanduc: Pentapeptide commonality between *Corynebacterium diphtheriae* toxin and the *Homo sapiens* proteome. *Immunotherapy* 3, 49-58 (2011)

51. SL Bavaro, M Calabro', D Kanduc: Pentapeptide sharing between *Corynebacterium diphtheriae* toxin and the human neural protein network. *Immunopharmacol Immunotoxicol* 33, 360-372 (2011)

52. D Kanduc: Immunogenicity in peptide-immunotherapy: from self/nonself to similar/dissimilar sequences. *Adv Exp Med Biol* 640, 198-207 (2008)

53. D Kanduc: HCV. Written in our DNA. *Self Nonself* 2, (2011) in press

54. D Kanduc: The self/nonself issue: a confrontation between proteomes. *Self Nonself* 1, 255-258 (2010)

55. D Kanduc: Correlating low-similarity peptide sequences and allergenic epitopes. *Curr Pharm Des* 14, 289-295 (2008)

56. D Kanduc: Protein information content resides in rare peptide segments. *Peptides* 31, 983-988 (2010)

57. PG Schoofs, HM Geysen, DC Jackson, LE Brown, XL Tang, DO White: Epitopes of an influenza viral peptide recognized by antibody at single amino acid resolution. *J Immunol* 140, 611-616 (1988)

58. S Tanabe: Epitope peptides and immunotherapy. *Curr Protein Pept Sci* 8, 109-118 (2007)

59. W Zeng, J Pagnon, DC Jackson: The C-terminal pentapeptide of LHRH is a dominant B cell epitope with antigenic and biological function. *Mol Immunol* 44: 3724-3731 (2007)

60. G Lucchese, A Stufano, B Trost, A Kusalik, D Kanduc: Peptidology: short amino acid modules in cell biology and immunology. *Amino Acids* 33, 703-707 (2007)

61. JB Rothbard, ML Gefter: Interactions between immunogenic peptides and MHC proteins. *Ann Rev Immunol* 9, 527-565 (1991)

62. MJ Reddehase, JB Rothbard, UH Koszinowski: A pentapeptide as minimal antigenic determinant for MHC class I-restricted T lymphocytes. *Nature* 337, 651-653 (1989)

63. B Hemmer, T Kondo, B Gran, *et al*: Minimal peptide length requirements for CD4(+) T cell clones--implications for molecular mimicry and T cell survival. *Int Immunol* 12, 375-383 (2000)

64. R Tiwari, J Geliebter, A Lucchese, A Mittelman, D Kanduc: Computational peptide dissection of Melan-a/MART-1 oncoprotein antigenicity. *Peptides* 25, 1865-1871 (2004)

65. EM Veys, P Hermanns, G Goldstein, P Kung, J Schindler, J Van Wauwe: Determination of T lymphocyte subpopulations by monoclonal antibodies in rheumatoid arthritis. Influence of immunomodulating agents. *Int J Immunopharmacol* 3, 313-319 (1981)

66. W Diezel, SR Waschke, K Forner: Induction and augmentation of mitogen-induced immune interferon production in human lymphocytes by a synthetic thymopoietin pentapeptide. Biomed Biochim Acta 43, 9-12 (1984)

67. W Roszkowski, J Stachurska, B Gerdin, T Saldeen, M Kopec: Suppression of cell-mediated immune reactivity by peptides cleaved from human fibrinogen. Ups J Med Sci 90, 279-291 (1985)

68. IZ Siemion, E Nawrocka, J Slon, A Pedyczak, A Kubik, K Spiegel, M Zimecki, Z Wieczorek: Immunoregulatory activity of substance P fragments. Mol Immunol 27, 887-890 (1990)

69. Z Szewczuk, A Wilczyński, P Stefanowicz, W Fedorowicz, IZ Siemion, Z Wieczorek: Immunosuppressory mini-regions of HLA-DP and HLA-DR. Mol Immunol 36, 525-533 (1999)

70. P Stefanowicz, PJ Boratynski, A Staszewska, A Wilczynski, M Zimecki, Z Szewczuk: Threonine at position 6 is not essential for the immunosuppressive activity of HLA-DQ[beta164-172]-hexapeptide. Mol Immunol 41, 911-917 (2004)

71. R Silva-Garcia, I Estrada-Garcia, R Ramos-Payan, A Torres-Salazar, ME Morales-Martinez, D Arenas-Aranda, JA Gimenez-Scherer, F Blanco-Favela, MG Rico-Rosillo: The effect of an anti-inflammatory pentapeptide produced by Entamoeba histolytica on gene expression in the U-937 monocytic cell line. Inflamm Res 57, 145-150 (2008)

72. DY Li, ZR Geng, HF Zhu, C Wang, DN Miao, PY Chen: Immunomodulatory activities of a new pentapeptide [Bursopentin] from the chicken bursa of Fabricius. Amino Acids 40, 505-515 (2011)

73. CA Janeway Jr: Approaching the asymptote? Evolution and revolution in immunology. *Cold Spring Harb Symp Quant Biol* 54, 1-13 (1989)

74. CK Fraser, KR Diener, MP Brown, JD Hayball: Improving vaccines by incorporating immunological coadjuvants. *Expert Rev Vaccines* 6, 559-578 (2007)

75. A Wack, BC Baudner, AK Hilbert, I Manini, S Nuti, S Tavarini, H Scheffczik, M Ugozzoli, M Singh, J Kazzaz, E Montomoli, G Del Giudice, R Rappuoli, DT O'Hagan: Combination adjuvants for the induction of potent, long-lasting antibody and T-cell responses to influenza vaccine in mice. *Vaccine* 26, 552-561 (2008)

76. E Tritto, F Mosca, E de Gregorio: Mechanism of action of licensed vaccine adjuvants. *Vaccine* 27, 3331-3334 (2009)

77. S Havarinasab, KM Pollard, P Hultman: Gold- and silver-induced murine autoimmunity requirement for cytokines and CD28 in murine heavy metal-induced autoimmunity. *Clin Exp Immunol* 155, 567-576 (2009)

78. CS Schmidt, WJ Morrow, NA Sheikh: Smart adjuvants. *Expert Rev Vaccines* 6, 391-400 (2007)

79. M Mizrahi, G Lalazar, A Ben Ya'acov, DM Livovsky, Y Horowitz, L Zolotarov, R Adler, D Shouval, Y Ilan: Beta-glycosphingolipid-induced augmentation of the anti-HBV immune response is associated with altered CD8 and NKT lymphocyte distribution: a novel adjuvant for HBV vaccination. *Vaccine* 26, 2589-2595 (2008)

80. T Yokochi, M Fukada, M Kawai, YH Zhang, GZ Jiang, K Takahashi: Novel adjuvant action of lipopolysaccharides that possess mannose homopolysaccharides as O-specific polysaccharides on immune responses to nonimmunogenic autoantigens in mice. *Infect Immun* 60, 4953-4956 (1992)

81. S Williamson: Anti-vaccination leagues. *Arch Dis Child* 59, 1195-1196 (1984)

82. TP Weber: Alfred Russel Wallace and the antivaccination movement in Victorian England. *Emerg Infect Dis* 16, 664-668 (2010)

83. G Scarpelli: "Nothing in nature that is not useful". The anti-vaccination crusade and the idea of *'harmonia naturae*' in Alfred Russel Wallace. *Nuncius* 7, 109-130 (1992)

84. A Beck: Issues in the anti-vaccination movement in England. *Med Hist* 4, 310-321 (1960)

85. D Kanduc: Peptimmunology: immunogenic peptides and sequence redundancy. *Curr Drug Discov Technol* 2, 239-244 (2005)

86. D Kanduc: Defining peptide sequences: from antigenicity to immunogenicity through redundancy. *Curr Pharmacogenomics* 4, 33-37 (2006)

87. D Kanduc: Epitopic peptides with low similarity to the host proteome: towards biological therapies without side effects. *Expert Opin Biol Ther* 9, 45-53 (2009)

88. D Kanduc, L Tessitore, G Lucchese, A Kusalik, E Farber, FM Marincola: Sequence uniqueness and sequence variability as modulating factors of human anti-HCV humoral immune response. *Cancer Immunol Immunother* 57, 1215-1223 (2008)

89. L Polimeno, A Mittelman, L Gennero, A Ponzetto, G Lucchese, A Stufano, A Kusalik, D Kanduc: Sub-epitopic dissection of HCV E1315-328HRMAWDMMMNWSPT sequence by similarity analysis. *Amino Acids* 34, 479-484 (2008)

90. A Lucchese, A Mittelman, L Tessitore, R Serpico, AA Sinha, D Kanduc: Proteomic definition of a desmoglein linear determinant common to *Pemphigus vulgaris* and *Pemphigus foliaceous*. *J Transl Med* 4, 37 (2006)

91. G Angelini, D Bonamonte, A Lucchese, G Favia, R Serpico, A Mittelman A, S Simone, AA Sinha, D Kanduc: Preliminary data on *Pemphigus vulgaris* treatment by a proteomics-defined peptide: a case report. *J Transl Med* 4, 43 (2006)

92. D Kanduc, R Serpico, A Lucchese, Y Shoenfeld: Correlating low-similarity peptide sequences and HIV B-cell epitopes. *Autoimmun Rev* 7, 291-296 (2008)

93. G Lucchese, A Stufano, D Kanduc: Proteome-guided search for influenza A B-cell epitopes. *FEMS Immunol Med Microbiol* 57, 88-92 (2009)

94. A Lucchese, R Serpico, V Crincoli, Y Shoenfeld, D Kanduc: Sequence uniqueness as a molecular signature of HIV-1-derived B-cell epitopes. *Int J Immunopathol Pharmacol* 22, 639-646 (2009)

95. G Lucchese, A Stufano, M Calabro', D Kanduc: Charting the peptide crossreactome between HIV-1 and the human proteome. Front Biosci 3, 1385-1400 (2011)

96. G Lucchese, A Stufano, D Kanduc: Searching for an effective, safe and universal anti-HIV vaccine: Finding the answer in just one short peptide. *Self Nonself* 2, 49-54 (2011)

97. D Kanduc: "Self-nonself" peptides in the design of vaccines. *Curr Pharm Des* 15, 3283-3289 (2009)

Key Words: Microbial immunoevasion, Microbial-vs-human peptide commonality, Peptide cross-reactivity, Autoimmune diseases, Sequence uniqueness, Safe vaccines, Review

Send correspondence to: Darja Kanduc, Department of Biochemistry and Molecular Biology, University of Bari, Bari, Italy, Tel.: 390805443321, Fax: 390805443317, E-mail: d.kanduc@biologia.uniba.it