Discovery of New Elements of Biological Communication Leading the Way to the Abolition of Infectious Diseases, Cancer, and Other Diseases as Causes of Human Mortality

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"Discovery consists in seeing what every body else has seen and thinking what nobody has thought." Albert Szent-Gyorgyi

Abstract

A new category of signals for biological communication has been discovered. Within a protein sequence one or more oligopeptides with a characteristic combination of conformation and charge function as potential signals for protein action. These signal oligopeptides represent the hydrophilicity maxima of a given protein. Moreover, these signal sequences are identical with the antigenic determinants of this protein. Antibodies binding to this protein reduce or block its metabolic interaction. Consequently, a new definition of antibodies, T cell receptors and related mediators of immune response is introduced: their general function is the interception of specific metabolic communication pathways. Signal oligopeptides as promoters of biological communication and immune response mediators as interceptors of these communication pathways form a diversified network modulating metabolic interactions. A rapid diversification of this biological communication network constituted the metabolic base for the differentiation of the human body and brain during human evolution. Of particular importance are the therapeutic implications of these discoveries. Signal peptides mediate the pathogenesis of a multitude of diseases. Synthetic analogs to one or more of these signal peptides can be used in acute as well as chronic therapy. Given intravenously, signal peptide analogs can competitively inhibit pathological communication. Given as a vaccine, these peptides can stimulate the production of antibodies which intercept pathologic communication and thereby disease progression. This publication provides the rationale for deciphering the communication code for human diseases. Its successful exploitation will lead to the abolition of infectious diseases, cancer, and many other diseases as causes for human mortality.

Introduction

In an earlier publication I had paved the way for the abolition of cardiovascular disease as a cause for human mortality.[1] In a subsequent paper I had presented the solution to the puzzle of human evolution.[2] The guiding molecule for these break-through discoveries has been apoprotein(a) [apo-a] and my quest to identify its physiological role as well as its metabolic regulation. In a recent paper I introduced potential signal sequences as mediators of the versatile metabolic actions of the apo-a molecule.[3] In this paper I will further elucidate this discovery and present a new category of biological signals as the long-sought missing link of biological communication.

Proteins mediate a multitude of biological functions including growth, differentiation, catalysis, and many others. Presently known categories determining protein action include conformation (such as helices, pleated sheets, disulfide bonds), charge (cationic or anionic regions within the protein sequence) and possibly carbohydrate attachment. It is evident that these coarse signal categories cannot explain the multitude of sophisticated biological messages and protein actions. The unique feature of the biological signals introduced here is a characteristic combination of two signal categories, conformation and charge, within the same oligopeptide sequence. These signal oligopeptides are proposed as important mediators for protein action and as promoters of biological communication in general. Other elements of this differentiated net-

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work of biological communication are signal interceptors. Antibodies and mediators of cellular immune response will be presented as interceptors of specific biological messages. Finally, the far-reaching therapeutic implications of these discoveries will be discussed.

Simple Elements of Protein Communication

A well-characterized signal sequence of protein communication is the tripeptide RGD composed of the cationic amino acid arginine (R), the anionic amino acid aspartate (D) and the neutral spacer residue glycine (G). The opposite charges of the RGD tripeptide sequence attract each other, resulting in a specific structural bending of this amino acid sequence. The resulting structure-charge pattern constitutes a simple but strong biological signal which can interact with a complementary conformation present in other proteins.

RGD has been shown to be involved in a multitude of biological interactions in human health and disease.[4] However, -little or no data are available for analogous tripeptides such as arginine-glycine-glutamate (RGE), lysine-glycine-aspartate (KGD) and others (Figure 1). I propose that these tripeptides also function as mediators for biological communication analogous to the.RGD tripeptide.

It has not escaped my attention that the cationic amino acids arginine and lysine, the anionic amino acids aspartate and glutamate, as well as the neutral amino acid glycine can all be encoded by purine nucleotides without requiring any pyrimidine nucleotides. This fact may indicate that RGD and the analogous tripeptides shown in Figure 1 are not only simple elements of protein language, but may also constitute early elements of biological communication during evolution.

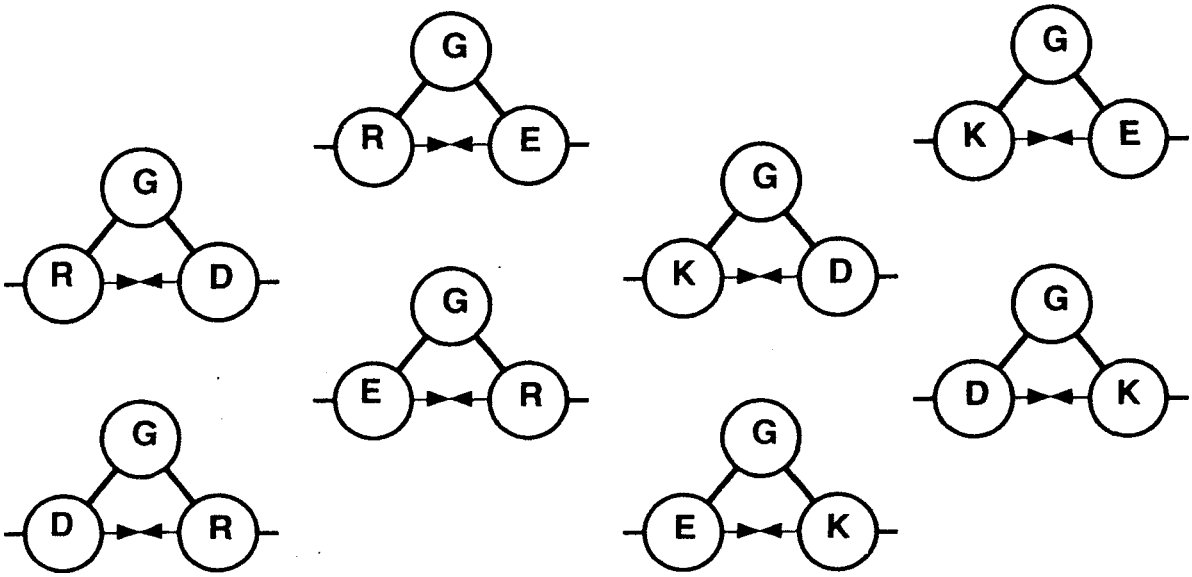

Figure 1. Simple words of protein communication. The cationic-anionic tripeptide RGD is a well characterized signal sequence for biological communication. It is composed of the cationic residue arginine (R), the anionic residue aspartate (D) and the neutral spacer residue glycine. The charged residues attract each other (-> <-) and form a biological peptide consisting of a characteristic conformation and charge. Analogous tripeptide signal sequences are arginine-glycine-glutamate (RGE), lysine-glycine-aspartate (KGD), lysine-glycine-glutamate (KGE), aspartate-glycine-arginine (DGR), glutamate-glycine-arginine (EGR), glutamate-glycine-lysine (EGK), and aspartate-glycine-lysine (DGK). Additional signal tripeptides are conceivable by inclusion of histidine which can be positively charged or uncharged dependent on the local environment as well as by substitution of the glycine spacer residue with another neutral amino acid.

Oligopeptide Signal Sequences Enriched in Charged Amino Acids as Differentiated Elements of Protein Action

It is evident that the limited number of charged tripeptide signals alone cannot fulfill the requirements of a differentiated biological language. I therefore postulate that tetrapeptides, pentapeptides, hexapeptides, and oligopeptides in general enriched in charged amino acids, form a characteristic conformation-charge element which can be recognized by complementary sequences in other proteins (Figures 2A and 2B). One or more oligopeptides composed of charged

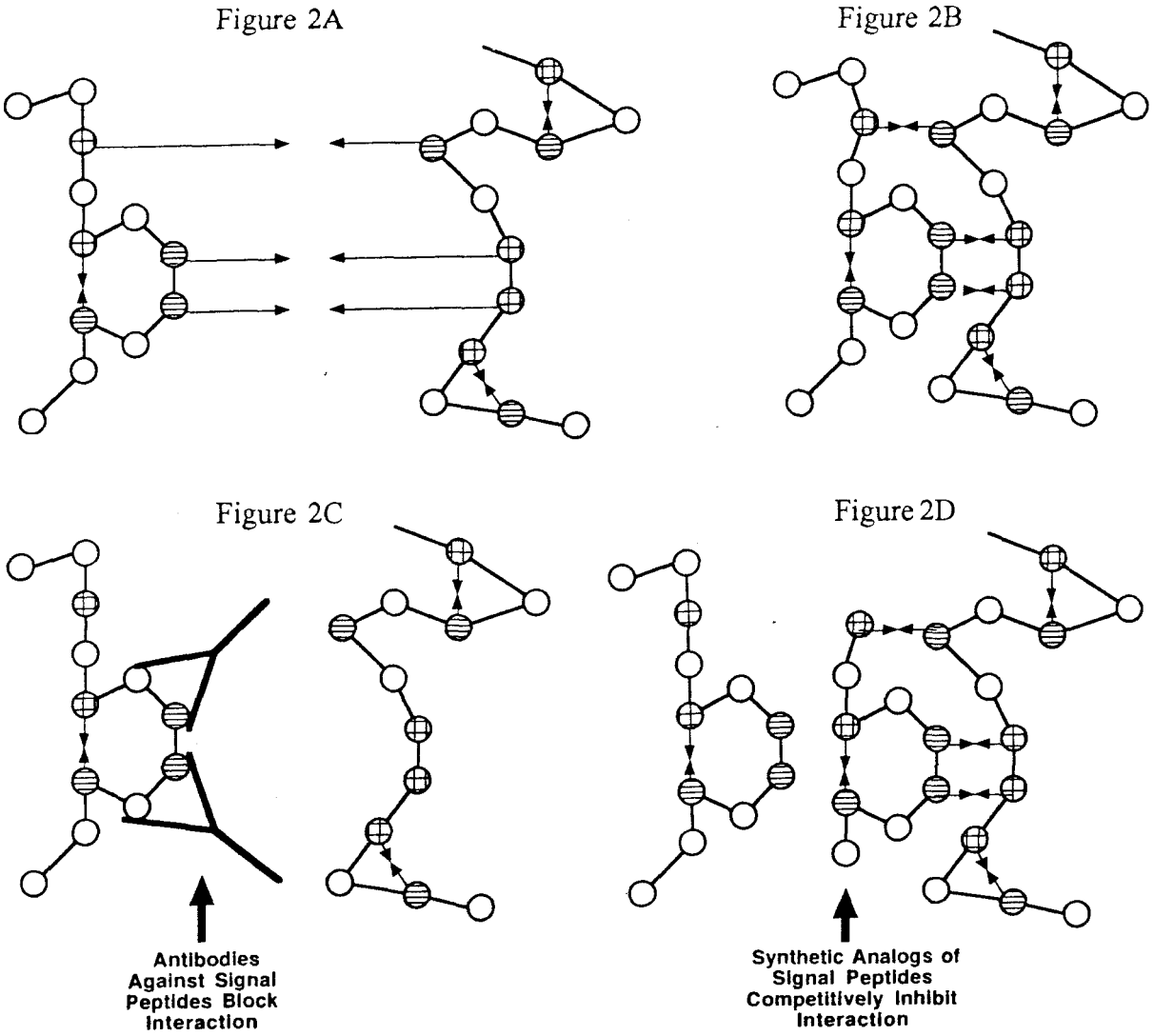

Figure 2. Diversified biological communication by oligopeptides with a characteristic conformation-charge signal. Oligopeptides analogous to the tripeptides shown in Figure 1 can function as mediators of differentiated biological communication. Figure 2A: The type and number of charged residues and their specific arrangement with non-charged residues forms a characteristic biological signal. Figure 2B: Via one or more of these signal oligopeptides a given protein interacts with complementary recognition sites of other proteins. This discovery can be used therapeutically in two principal ways. Figure 2C: In chronic therapy synthetic analogs to specific signal oligopeptides can be used as a vaccine to stimulate the production of antibodies and cellular immune responses capable of blocking specific pathogenic communication pathways. Figure 2D: In acute therapy synthetic analogs to signal peptides can be applied intravenously to competitively inhibit a specific pathological interaction. (+) Cationic amino acid residues and (-) anionic amino acid residues are shown schematically.

amino acids in a variable arrangement with non-charged amino acids within a protein are the biological signals of this protein and they are designated hereafter signal oligopeptides. The amino acid proline, which may enhance the signal character of these oligopeptides due to its bending properties, and charged amino acids may be similarly important for the signal function of these oligopeptides.

Signal oligopeptides in a given protein can be identified with reasonable approximation by identifying the hydrophilicity maxima of the protein sequence.[4] Hydrophilic oligopeptides enriched in charged amino acids and proline constitute a huge reservoir of possible combinations which meet the requirement for differentiated biological communication. In some proteins RGD and analog tripeptides are found in addition to a longer signal oligopeptide. It is proposed that in this case signal tripeptides serve as strong primary anchors whereas more complex signal oligopeptides mediate the differentiated action of this protein.

Particularly enriched in these signal oligopeptides are adhesive proteins, adhesion molecules, acute phase proteins, coagulation factors, and certain other proteins. The enrichment of these proteins in signal sequences may reflect a particular need for optimum precision and timing of biological communication during situations of metabolic alert.

Letters, Words, and Sentences of Protein Communication

Essential elements of protein language are biological letters, words, and sentences. These elements of biological language are exemplified here for the interferon alpha (INFa) family. Figure 3 shows the sequence alignment of residues 31 to 52 among certain members of the INFa family.

Sentences of protein communication

The complete structure of a protein is the sentence in the language of protein communication and is ultimately responsible for its precise biological message and its action.

Residue:	31	40	50
IFN-α consensus	K D R H D F G F P Q E E F D G N Q F Q K A Q		
IFN-αK (α6)	- - - - - - R - - - - - - - - - - - - - E		
IFN-αH1 (αH2)	- - - - - - E - - - - - - - - - - - - - -		
IFN-αB2 (α8)	- - - - - - E - - - - - - - D K - - - - - -		
IFN-α4b	- - - - - - - - - E - - - - - H - - - - - -		
IFN-αC	- - - - - - R I - - - - - - - - - - - - -		
IFN-αJ1(α7)	- - - - - E - R - E - - - - - H - - - - - -		

Figure 3. Letters, words, and sentences of protein communication - exemplified for the interferon-a (IFNa) family. Letters words and sentences compose the language of protein communication. The complete three dimensional structure of a protein constitutes the sentence within the language of protein communication which ultimately determines the action of this protein. Within this sentence one or more signal oligopeptides constitute the words and the amino acids represent the letters of protein language. Substitution of a single charged amino acid can modulate the action of the whole protein by changing conformation and/or charge of a signal oligopeptide. Figure 3 represents the sequence alignment of residue 30 to 52 of selected members of the IFNa family, only substituted amino acid residues are shown. Note that almost all substituted residues within this sequence are charged. The conventional one-letter symbols of amino acids are used.

Words of protein communication

Signal oligopeptides are the words of protein language. These words give the sentence a specific meaning. Modification of a signal oligopeptide (word) modulates the action of the protein (sentence).

Letters of protein communication

The letters of protein communications are the amino acids. The substitution of one single residue (letter) within a signal oligopeptide (word) can modulate the action of the protein (sentence). It is therefore no surprise that substituted amino acids are frequently charged, implying that modulation of signal oligopeptides may be a preferential means of differentiated biological messages. The substitution of single charged amino acids may have evolved as a particularly economic way of nature to modulate biological messages and diligently modify protein action, as exemplified here for the INFa family (Figure 3).

In the following paragraphs I will discuss the versatile interaction of signal oligopeptides as promoters of biological communication and their interaction with antibodies as specific interceptors of biological communication.

Antibodies and Mediators of Cellular Immune Response Are Specific Interceptors of Biological Communication

It has been observed that the hydrophilic maxima within a protein sequence constitute the antigenic determinant of this protein.[5] One of the most important discoveries presented in this paper is the fact that the antigenic sites of a protein correspond with the signal oligopeptide sequences for biological communication. The hydrophilicity maxima therefore determine both the antigenic determinants as well as the biological signal sequences. This discovery leads to a new definition for the biological function of mediators of humoral and of cellular immune response: immunoglobulins, T cell receptors, major histocompatibility complex (MHC) proteins and related mediators of immune response are specific interceptors of biological communication. The general role of antibodies and analog immune response mediators is therefore to block specific biological messages. I have not found any earlier description of this concept in the scientific literature.

The definition of immune response mediators as interceptors of biological communication is readily conceivable for infectious diseases where these molecules block the bib-logical action of pathogens. The generality of this concept, however, implies its validity also for atherosclerosis, cancer, and other diseases. It is a well known fact that patients with metastatic cancer frequently have kidney complications due to the increased presence of immune complexes in their circulation. Certain proteins involved in tumor promotion and metastasis evidently serve as antigens and thereby trigger the immune response. Similar observations, to a varying degree, have been made in atherosclerosis and many other diseases. Although clinical reports for an immunological involvement are available for a multitude of human diseases, the underlying rationale for these observations is insufficiently - or not all - understood. The rationale is as follows: certain oligopeptide signal sequences play a decisive role as mediators of disease progression and at the same time trigger a specific immune response.

Hypervariable regions of immunoglobulins are enriched in charged amino acids

Hypervariable regions in the variable domains of both the light and the heavy chains of immunoglobulins determine the specificity of the antibody combining site with the antigen and are known to vary considerably from one antibody to the next .[6] My preliminary screening of hypervariable regions in a series of variable regions of immunoglobulin light and heavy chains revealed particular enrichment in charged amino acids. The abundant presence of charged amino acids in the hypervariable regions of immunoglobulins constitutes strong support for the concept presented in this paper. The hypervariable regions of immunoglobulins thus represent an ideal reservoir for studying the interaction of signal oligopeptides with complementary peptide sequences.

In summary, signal oligopeptide sequences are promoters of biological communication and certain members of the immunoglobulin gene superfamily are interceptors of biological communication.

The Immunoglobulin Gene Superfamily

The immunoglobulin gene superfamily is named for the immunoglobulin light- and heavy chain gene families and comprises a series of gene families including immunoglobulins, T-cell receptors, MHC proteins, and other representatives with primarily immune response functions. Surprisingly, this family also comprises a group of proteins mediating cell-cell interaction and other forms of adhesion. This group includes intercellular adhesion molecules (ICAMs), vascular cell adhesion molecules (VCAMs), neuronal cell adhesion molecules (NCAMs) and other adhesion molecules and adhesive proteins. Many of these proteins are enriched in signal oligopeptides, facilitating their interaction with cellular and extracellular constituents. Until now no explanation has been offered as to why proteins of so different functions as immune response and adhesion belong to the same gene family. The concept presented here can provide this answer. Both functions, immunological response and adhesion, are critical features of biological communication and play an important role during growth and differentiation of organisms as well as during evolution.

Differentiated Biological Communication as a Driving Force of Human Evolution

Evolution is characterized by differentiation and diversification of biological communication and metabolic interaction. These processes were particularly important during the evolution of the human brain and body in the relatively short time in which man became the dominant species on earth. In a recent publication I have explained human evolution as the result of a fascinating combination of genetic, metabolic, dietary, and environmental factors.[2]

The genetic precondition for the evolution of man was the loss of endogenous ascorbate production which set the metabolism of humans fundamentally apart from that of other mammals. This genetic precondition was unmasked during the ice ages when exogenous ascorbate supply during millennia approximated zero and scurvy became the greatest threat to the evolutionary survival of man. Scurvy is characterized by a multitude of metabolic dysfunctions, the most relevant of which are the structural impairment of the body and impairment of the immunological defense. Ascorbate deficiency decreases collagen synthesis and leads to destabilization of the extracellular matrix throughout the body, to increased fragility of the blood vessel wall, scorbutic blood loss, increased susceptibility to internal and external wounds, and to impaired wound healing. Ascorbate deficiency also leads to decreased immunological defense due to dysfunction of lymphocytes and to other impairments of immunological responses.

Metabolic features counteracting any of these major challenges caused by ascorbate deficiency had a selective evolutionary advantage. Adhesive proteins (apo-a etc.), adhesion molecules (ICAMs, NCAMs etc.) as well as growth factors gained an evolutionary advantage due to their compensatory function in tissue repair and wound healing. At the same time the increased susceptibility to infectious diseases triggered the diversification of the immune system. Evolutionary survival became dependent on an improved metabolic communication and promoters and interceptors of biological signals were greatly favored. Both of these signal categories are represented in the immunoglobulin gene superfamily and it is readily conceivable that the members of this gene family and their diversification were of particular importance for the evolutionary survival of our ancestors. I had proposed earlier that the greater the selective advantage of genetic and metabolic features was in counteracting the life-threatening consequences of scurvy during the evolution of man, the greater was its contribution to the differentiation of the human body and brain *}* The immunoglobulin gene superfamily thus represents a versatile group of potential metabolic promoters of evolution (Figure 4). This concept fundamentally questions the clonal selection theory of immunology according to which the combining site of an antibody molecule is completely determined before it ever encounters an antigen. Increased susceptibility to infection, particularly during the millennia of glaciation, provided the evolutionary pressure for extension and differentiation of immunoglobulin genes and the immune system in general. The clonal selection theory thus has to be revised for the simple fact that it is not compatible with the genetic differences in the immune system between

different species and because it essentially negates evolution.

The concept presented in the previous paragraphs has immediate therapeutic implications.

A New Category of Therapeutic Options. The identification of signal oligopeptides as promoters and of immune response mediators as interceptors of biological communication leads the way to a new category of therapeutic options in many areas of clinical medicine. Synthetic analogs to signal peptides can be therapeutically used in two principal ways: first, in acute therapy as a competitive inhibitor of pathological communication, in which

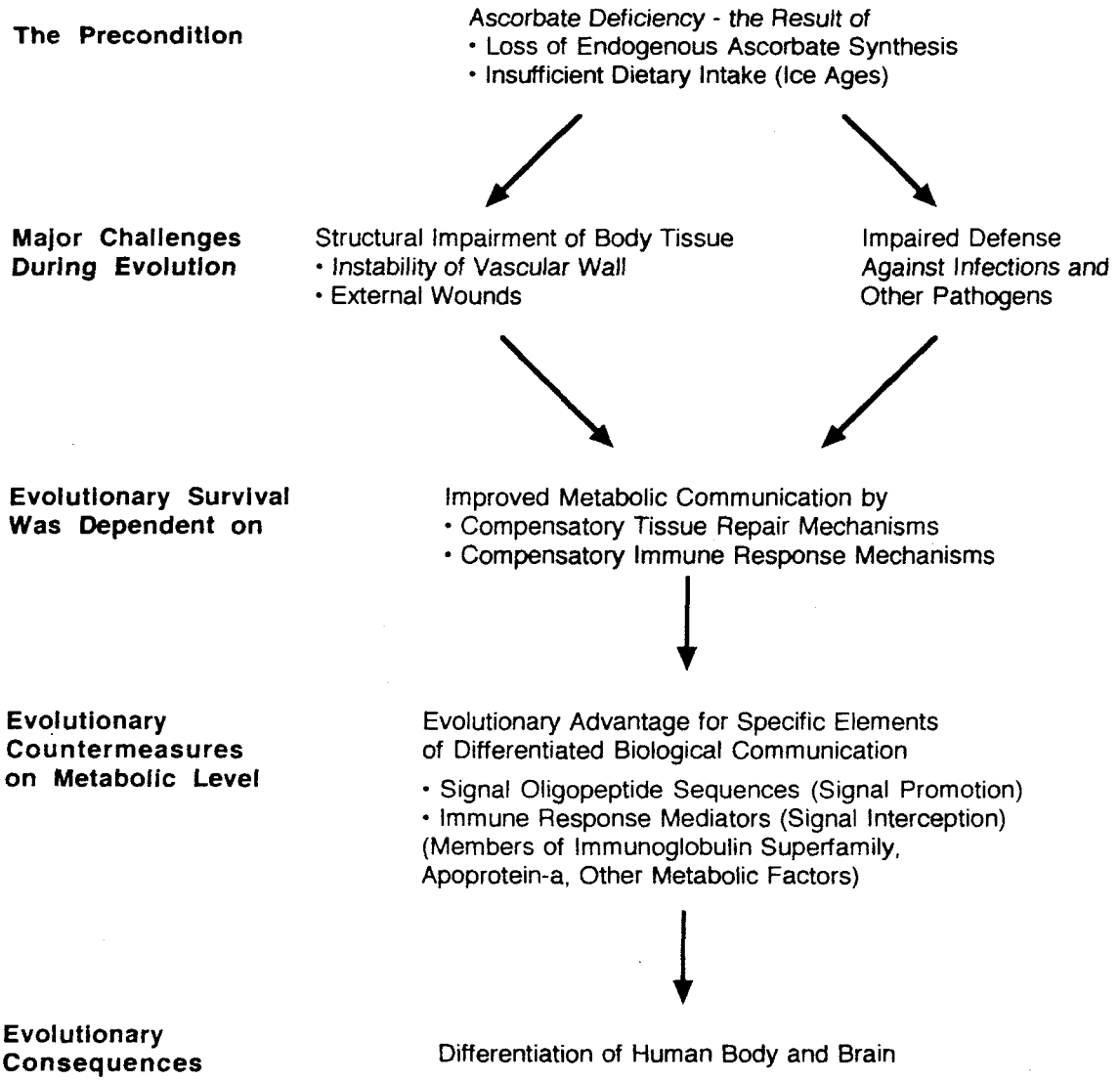

Figure 4. Diversification of biological communication as the driving force of human evolution. Human evolution was triggered by the inability of our ancestors for endogenous ascorbate synthesis in combination with an exogenous ascorbate supply approximating zero during the ice ages. Extinction from scurvy became the greatest threat for the evolutionary survival of man. Genetic features compensating for the metabolic impairments caused by ascorbate deficiency had a selective advantage during the evolution of man. Genetic and metabolic features improving tissue repair, immune defense, and biological communication in general were of particular importance and constituted the genetic and metabolic base for the differentiation of the human body and brain.

case the synthetic oligopeptides would preferentially be applied intravenously. Second, in chronic therapy synthetic oligopeptides can be used as vaccines to stimulate the production of antibodies and other specific immune responses capable of intercepting pathological communication processes in a variety of diseases (Figure 2C and 2D).

Cancer

Synthetic analogs to signal oligopeptides of tumor promoters, membrane proteins, adhesive proteins, adhesion molecules, growth factors, to signal sequences of corresponding receptors, and to other proteins involved in tumor promotion and metastasis can be used therapeutically. In the context of this paper it is of interest that polymeric RGD has been shown to inhibit experimental metastasis.[8] However, the clinical application of poly-RGD is questionable since, as discussed above, the RGD tripeptide is a strong but rather nonspecific signal sequence and its ubiquitous competitive inhibition is likely to cause unde-sired side effects. In contrast, clinical use of more differentiated signal oligopeptides identified on the basis of this publication may lead to the long-sought breakthrough in the therapeutic control of cancer. The advances in cancer immunotherapy have recently been reviewed.[9] The observations reported are in striking congruency with the concept presented here.

Infectious Diseases

Synthetic analogs to signal oligopeptides of toxins, capsid proteins, and other proteins involved in the pathogenesis of infectious diseases can be therapeutically used.

Cardiovascular Disease

Synthetic analogs to signal sequences of apo-a, ICAMs, VCAMs, and other proteins, involved in the development of atheroascler-osis can be used in the therapy of cardiovascular diseases.

This publication also provides the rationale for yet unexplained therapeutic phenomena such as effective use of bacterial toxins in the treatment of cancer.[10] In this case the signal oligopeptides of toxins induce the production of antibodies which simultaneously block tumor growth.

It should be emphasized that the therapeutic use of synthetic signal oligopeptides is only the second stage in a differentiated clinical therapy. The first stage is the therapeutic effort to decrease the synthesis rate of proteins containing pathological signal sequences.

A New Therapeutic Goal is Defined: The Abolition of Infectious Diseases, Cancer, And Many Other Diseases as Causes of Human Mortality

Vitamins, minerals and other essential nutrients are important co-factors for a multitude of metabolic reactions. Many of these essential nutrients decrease the synthesis rate of proteins with potentially pathological signal sequences. In a recent paper I presented ascorbate and niacin as essential co-factors for decreasing the synthesis rate for apo-a." Similarly, ICAMs and other adhesion molecules will be shown to be co-regulated by ascorbate and other essential nutrients.

In summary, future clinical therapy will be based on two main categories. A first category comprises the preventive and therapeutic use of nutritional supplements. If necessary, a second therapeutic level is added in form of synthetic signal oligopeptides as inhibitors of specific pathological interactions (Figure 5). These therapeutic options will lead the way to the abolition of infectious diseases, cancer and many other diseases as causes of human mortality.

Conclusion

The discoveries reported in this paper will lead to the abolition of many human diseases in future generations of mankind. The 20th century may enter in the annals of medical history as an episode when therapeutic research frequently became deadlocked in the attempt to outsmart nature with artificial therapeutic approaches rather than to make use of the therapeutic options provided by nature itself.

A word of caution is in order. The technology revealed in this publication enables the manipulation of growth and differentiation of organisms including the human body with consequences similar to those of genetic engineering. The further exploitation of this new therapeutic technology therefore will require defined scientific as well as ethical guidelines.

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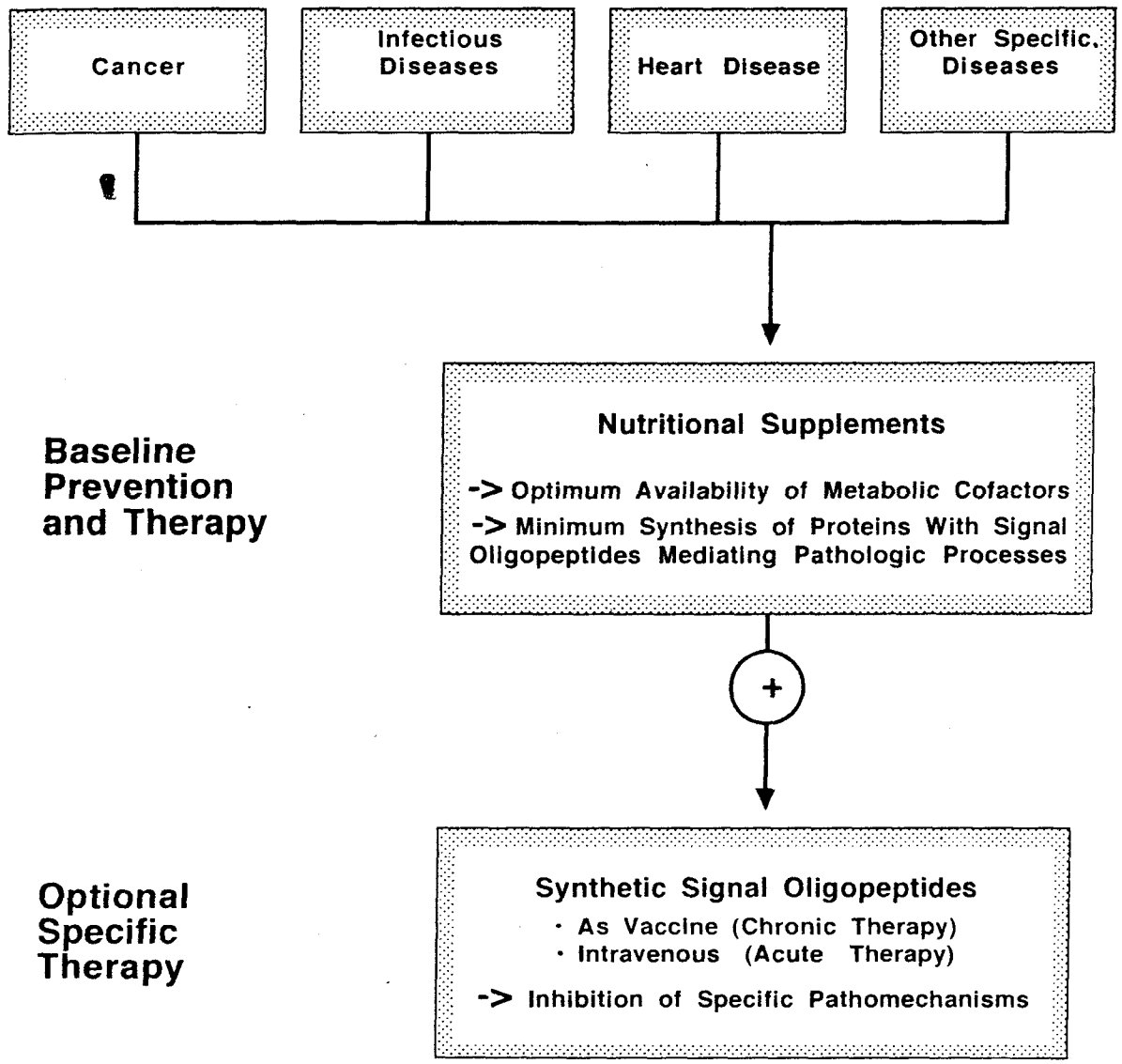

Figure 5. General scheme for prevention and treatment of human diseases in future generations. Future therapeutic options will have two main levels. The first level consists of nutritional supplements which function as metabolic co-factors and at the same time minimize the availability of proteins containing pathological signal sequences. If necessary, a second therapeutic level can be added. Synthetic analogs to signal oligopeptides can be used for the treatment of acute diseases as well as chronic diseases. A mixture of synthetic analogs to several specific signal sequences may be necessary to achieve the ultimate goal: the abolition of most of today's diseases as causes of mortality in future generations of mankind.

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