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A new take on comparative immunology; Relevance to immunotherapy

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Summary

It is becoming increasingly recognized that experimental animal models, while useful to address monothematic biological questions, bear unpredictable relevance to human disease. Several reasons have been proposed. However, the uncontrollable nature of human genetics and the heterogeneity of disease that with difficulty can be replicated experimentally play a leading role. Comparative immunology is a term that generally refers to the analysis of shared or diverging facets of immunology among species; these comparisons are carried according to the principle that evolutionarily conserved themes outline biologic functions universally relevant for survival. We propose that a similar strategy could be applied searching for themes shared by distinct immune pathologies within our own species. Identification of common patterns may outline pathways necessary for a particular determinism to occur such as tissue-specific rejection or tolerance. This approach is founded on the unproven but sensible presumption that Nature does not require an infinite plethora of redundant mechanisms to reach its purposes. Thus, immune pathologies must follow, at least in part, common means that determine their onset and maintenance. Commonalities among diseases can, in turn, be segregated from disease-specific patterns uncovering essential mechanisms that may represent universal targets for immunotherapy.

Keywords

Comparative immunology; tumor immunology; vaccines; chronic infection

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Experimental animal models poorly represent human pathology

There are several aspects of immunology that are shared by evolutionarily distant species. For instance, several growth factors such as Transforming Growth Factor (TGF)- β , Connective Tissue Growth Factor and Vascular Endothelial Growth Factor (VEGF) and several chemokines (chemotactic cytokines) such as CXCL-8/interleukin (IL)-8 and CCL2/monocyte chemo-attractant protein-1 are evolutionarily conserved in vertebrates and invertebrates⁽¹⁾. These molecules are involved in complex interplays during innate immune responses to pathogens leading, when successful, to disease clearance, tissue repair, angiogenesis and wound healing. When homeostatic mechanisms go awry, the same factors may be involved in the pathogenesis of chronic infections, autoimmunity or cancer^(2,3). Evidence that these and several other cytokines and growth factors are phylogenetically ancient^(4,5) suggest that these proteins are fundamental for the survival of multi-cellular organisms. Most of them played originally a role in embryonic development (invertebrates) and progressively acquired during evolution accessory homeostatic and defensive functions (vertebrates). Moreover, some innate immune defenses can be traced back in evolutionary history to invertebrates which share the expression of C3 complement components⁽⁶⁾ and Toll-like receptor signaling molecules⁽⁷⁾. Growth factor and cytokine function is often shared among vertebrates to the point that human molecules can be bioactive in some lower species such as human TGF- β which can affect the function of fish macrophages⁽⁸⁾. The opposite is also true, for instance, CXCL-8/IL-8 and its specific receptor (CXCR1) are not expressed by several mammalian species including mice^(9,10); in these species, IL-8 functions are believed to be carried, at least in part, by genetically close CXCL chemokines belonging to the same family (CXCL-1, -2, -3, -4, -6, -7) which bind the common and promiscuous CXCR2 receptor. This difference between species is, however, critical because, while interactions with the CXCR2 receptor mediate primarily pro-angiogenic effects, CXCR1 signaling is believed to be responsible for the pro-inflammatory functions of IL-8^(11,12). Yet, human IL-8 can induce bacterial phagocytic activity in the mussel suggesting that its role as modulator of innate immunity is ancient and might have been lost by some higher species during evolution but not by humans⁽¹³⁾. Thus, not surprisingly, aggressive human cancer cell lines when transplanted in nude mice seem to exercise their infiltrative and pro-angiogenic potential through the secretion of IL-8 which at the same time appears not to induce, at least in a model of orthotopic glioblastoma, any pro-inflammatory effects⁽¹⁴⁾.

Thus, while dramatic examples of conservation across species can be easily identified, it is clear that as a whole, each animal species has adapted to the peculiarities of its own environment following diverging strategies. These differences cannot be dismissed and suggest that simplistic translation of biological information obtained from a species to another is inappropriate⁽¹⁰⁾. Mice have been the experimental tool of choice of immunologists for several practical reasons; yet, particular attention should be placed in comparing data obtained from mice when moving from basic biology to modeling of complex human diseases⁽¹⁵⁾. As summarized by Davis MM⁽¹⁵⁾, it has been proposed that mice represent poor human models because *i)* the use of inbred strains induce a prevalence of homozygous recessive defects that may skew the regulation of the immune response⁽¹⁶⁾; this may be extremely important considering the lethal role that even some heterozygous deletions of cytokines can play in phenotype development⁽¹⁷⁾; *ii)* moreover, model systems of disease are often carefully and arbitrarily planned according to a specific biologic or therapeutic purpose; this is the opposite of human disease which serendipitously occurs as the independent variable and demands treatments to be tailored according to individual's needs⁽¹⁸⁾; *iii)* finally, as previously discussed, the million of years of evolutionary divergence among animals exposed to significantly different environmental challenges plays a role that cannot be dismissed⁽¹⁰⁾. Immune geneticists are well aware of the dramatic

effects that a single polymorphism within the human species can have on disease outcome. A good example is the $\Delta 32$ mutation of the CCR5 gene that offers complete protection to human immune-deficiency virus (HIV) infection⁽¹⁹⁾. It is naïve to believe that similarities but not identity among species and homologies among genes can be regarded as relevant in view of the human experience where single nucleotide variants can define divergent phenotypes. In our opinion, however, the primary reason for the unreliability of animal models as prototype of human disease, resides in the uncontrollable nature of human disease^(20,21). Humans are polymorphic, their diseases are heterogeneous and their environmental exposure complex and ever-changing. The goal of animal modeling is to minimize experimental variance and optimize experimental reproducibility by inbreeding the animals, cloning their tumor cells or selecting stable clades of pathogens in a controlled environment. However, this strategy causes a colossal experimental bias starting from the selection of “borderline” experimental models around which modifications are performed to shift the balance in favor of disease eradication⁽²²⁾. In a classic pre-clinical model, adoptive transfer of tumor infiltrating lymphocytes in combination with IL-2 ignited hope because of the successful treatment of advanced mouse cancers derived from carefully selected cancer cell lineages, inoculated in animals at an appropriately titered number and allowed to grow for a specific amount of time⁽²³⁾. The addition of cyclophosphamide to the treatment lead to 100% regression of tumors in treated animals⁽²⁴⁾. Unfortunately, these promising results in mice did not endure the challenge of human reality as only a small proportion of patients responded at a frequency comparable to that observed with the administration of IL-2 as single agent^(25,26). Yet, it could be predicated that if it could have been possible to clone a patient responding to that therapy into 100 identical siblings and at the same time tumor cells could be expanded from his tumor and reintroduced into his siblings, a response rate close to 100% might have been observed. Thus, by firming the bulls-eye in the trajectory of the bullet, perfect results can be expected⁽²⁰⁾. Unfortunately, this approach, while highly successful for academic purposes, does not take care of the realities of human suffering. In our opinion, the power to manipulate model systems compared to the uncontrollability of humans is the true essence of the discrepancy between pre-clinical models and clinical results. In fairness, recent work from the same group has greatly enhanced the effectiveness of therapy by the addition of other immune manipulations that greatly increased the effectiveness of treatment⁽²⁷⁾. Another striking example of discrepancy between promising pre-clinical data relatively poor clinical outcome is the treatment of mice with mucin-1 vaccine which has caused consisted anti-tumor responses in pre-clinical settings but little effectiveness in humans^(28,29). Attempts have been made to develop humanized mouse models that potentially may bear better relevance to human immune-related pathologies^(30,31,32), in addition, the recently described Vk myc model very closely mimics multiple myeloma with clinical features that are characteristic of human disease⁽³³⁾; thus, future efforts may improve the relevance of animal experimentation but a careful evaluation of the selected phenotype.

Thus, while comparative immunology provides evidence of shared adaptive strategies to environmental challenges, it also demonstrates vast differences existing among species. “Although all of us have a tendency to over generalize about the significance of results obtained from just a few species....we have to remember that model systems don't always represent all other species”⁽³⁴⁾. In summary, the study of immunology along the evolutionary process is informative only when individual patterns are shared. These shared evolutionary facets of immunology must be proven to exist among several species and, when proven, stand by themselves and cannot be used as proof of principle that other, unproven, pattern similarities should be all the same expected.

Impact of human polymorphism and disease heterogeneity on the immunotherapy of humans

The pathology of humans, in contrast to that of inbred laboratory animals faces the challenge of diversity addressed in genetic terms as polymorphism⁽³⁵⁾. Emerging evidence suggests that the study of complex systems such as the cytokine network is complicated by inter-individual differences related to increasingly recognized polymorphisms. Polymorphism appears widespread, particularly, among genes of the immune system possibly resulting from an evolutionary adaptation of the organism facing an ever evolving environment. The weight of the environment on the evolutionary adaptation of the human immune system is best exemplified by the work of Cavalli-Sforza and other geneticists who have tracked the anthropological variation of the major histocompatibility complex along migratory routes through the history of our species^(36,37,38). Yet, other species like the chicken adapted to their environment (Borneo from which they all originated) by modifying their major histocompatibility complex following a strategy completely different from that adopted by humans. The strategy adopted by chicken well-suited their survival in their ancestral environment but poorly adapted to their survival in farms throughout the world where inbred chicken species are decimated by viral epidemics⁽³⁹⁾.

Several examples demonstrate how polymorphism of immune genes may affect disease or treatment outcome; polymorphism of the IFN- γ gene may affect responsiveness of patients with advanced cancer to the systemic administration of IL-2⁽⁴⁰⁾; variants of CCR5 may affect the long term survival of patients with melanoma treated with immunotherapy⁽⁴¹⁾ while at the same time CCR5 polymorphism may affect the infectivity of HIV⁽¹⁹⁾; a mutation of the Fas gene can induce a complex lymphoproliferative syndrome associated with autoimmune pathology^(42,43,44); yet, the penetrance and morbidity of the disease in carriers of the mutation are different among members of the same family suggesting that the syndrome itself is dependent on other modifiers yet to be uncovered^(45,46). Several point mutations have been identified that are at the basis of recurrent inflammatory syndromes, a pro-inflammatory phenotype⁽⁴⁷⁾ or autoimmune diseases such as rheumatoid arthritis⁽⁴⁸⁾. These and other examples^(49,50,51,52) emphasize the increasing number of immune pathologies that are dependent upon single alterations of one or a few genes. The overall impression from these genetic studies is that most syndromes can be attributed to specific mutations linked to one or several independent genes with redundant functions, but the penetrance and clinical spectrum is dependent on a myriad of genetic modifiers yet to be determined. As an extrapolation, it could be guessed that a mosaic of variations among individuals may shape a sub-clinical diversity in immune responses whose global effect is the determination of a unique immune phenotype for each individual⁽³⁵⁾. The discovery for the potential of millions of variant genes within the human genome⁽⁵³⁾ has rendered the study of these interactions extremely complex clearly establishing the need to identify adequate strategies for the analysis of complex diseases in which a genetic influence is estimated to affect the outcome: these studies must consider complex relationships that modify an individual's susceptibility and include gene-to-gene interactions and their evolutionary relation to disease association^(54,55,51,56,57).

Human germ line variation combines with the extreme heterogeneity of human diseases and the rapidity in which they can evolve. Cancers, for instance, represent a myriad of diseases clustered according to the tissue of origin and sub-categorized according to the ocular resolution of their histological appearance. However, global transcriptional profiling has proposed distinct molecular taxonomies among histologically identical cancers^(58,59,60) that can further subcategorize the disease. Moreover, molecularly-defined patterns clearly demonstrate significant patient-specific divergence within each molecular classification of cancer^(61,62,63,64). Although it remains to be conclusively established whether distinct cancer

phenotypes represent distinct molecular taxonomies rather than evolving aspects of the same disease⁽⁶²⁾, it is clear that each individual with cancer is facing her/his own unique challenge and the same will have to be faced by her/his own immune system. It is likely that, because of the extreme genetic instability of cancer^(65,66,67), the immune system is confronted with a disease in fast lane evolution^(68,69,70). Although a relative genetic stability of clonal cancers such as metastatic melanoma can be easily demonstrated, dramatic changes in genetic imbalances and consequently in phenotype could occur with time that completely alter the immunogenic and biologic potential of each individual's cancer^(63,64). In fact, genetic alterations stem from a core of genetic stability through a random and non-sequential process that proceeds independently in individual metastases to the point that even synchronous lesions can display divergent⁽⁶¹⁾ and some time opposite⁽⁷¹⁾ phenotypes. Such evolutionary variations are clearly increased in frequency and rapidity of occurrence when strong immune pressure is applied by successful treatments^(72,73,74). Pathogens, particularly RNA viruses are well-known for their genetic instability⁽⁷⁵⁾; great examples are the variation of hepatitis C virus (HCV) and the human immune deficiency virus (HIV) under immune selection^(76,77,78).

Thus, the complexity of human disease determined by the genetic background of the host, heterogeneity and rapid evolution of disease and uncontrollable environmental pressure is at the basis of its erratic responsiveness to treatment making it impossible to address in a systematic monothematic way the determinism of each individual patient's sickness. Following an alphabetical or numerical list to address individual genes and pathways one at the time and their relationships with each other would not be the smartest way to identify the only ones that really matter for a occurrence of a particular phenomenon: yet, this is what most of us are currently doing.

Non-linear mathematics applied to human disease

A child asked what makes the rain fall will likely point at the clouds. Truly, rain falls from clouds, but this answer does not explain why the clouds are there and, therefore, the reason(s) for the rain. A meteorologist would argue that atmospheric pressure, oceanic currents, evaporation, jet streams, environmental pollution, Earth's rotational axis, all combined cause the condensation of water in the sky and its movements to create those patterns that from the ground we call clouds. An astronomer would, additionally, argue that the tilt of Earth's orbit around the sun changes periodically throughout the millennia influencing, in turn, temperature changes and the global environment. At tumor immunology meetings, scientists argue that tumors grow because T regulatory cells counteract immune effector functions, others blame myeloid regulatory cells or immune suppressive cytokines, and others metabolic bio-products⁽⁷⁹⁾; yet few ask themselves why are those cells and/or regulatory factors there in the first place⁽⁷⁴⁾. Like children pointing at the clouds, we do not know which, when taken alone, has anything to do with tumor growth and why. For this reason, we have long argued that the study of complex diseases such as cancer and its interaction with its host requires a global approach using high-density analytical tools that could provide an unrestricted and less speculative view derived directly from human observation as a reverse of the standard bench-to-bedside model^(20,80,81,82). This bottom-up, inductive approach is aimed at identifying facts upon which we can firm our hypotheses over the solid based of human reality⁽²²⁾. Such approaches are often derided as “descriptive” or “not hypothesis driven”; however, they have the indisputable advantage of providing information derived from observations performed at the right moment and at right place which, returning to the previous aphorism, would correspond to the recognition even by our Neanderthal relatives that clouds in one way or another had something to do with the rain. In global terms, this inductive approach could be assimilated to the study of weather patterns for forecasting purposes^(83,84,85,86,87). Thus, facts-derived observations (also referred to as

inductive reasoning or bottom-up investigation) may, in turn, provide novel hypotheses more likely to be relevant to human disease⁽⁸⁸⁾.

Complex human diseases can only be studied by a global approach: as previously discussed, monothematic, gene-specific, hypothesis-driven, deductive approaches cannot encompass the complexity of humans; this is because humans have heterogeneous genetic background; they are exposed to uncontrollable environmental forces, their diseases often stem from multi-genic causes and are rapidly evolving^(20,21,22). Thus, a paradigm shift is needed for the way research is executed in humans that includes two components: 1) The application of inductive reasoning; 2) a global approach to the study of humans.

Deductive versus inductive reasoning

The current scientific establishment much prefers deductive reasoning for the pursuit of the scientific truth⁽²¹⁾. Deductive reasoning follows a top-down process in which hypotheses are generated through in depth analysis of archival information with the expectation that an effective transition from the known to the unknown could be achieved through the ingenuity and intuition of the investigator. When the hypothesis is generated, its validation depends upon the execution of the third Baconian principle of the scientific revolution: hypothesis testing⁽⁸⁹⁾. In turn, hypothesis testing can only be done by minimizing experimental variance to one variable (gene/protein/interaction) at the time rigorously controlling the other conditions. As previously discussed, this approach poorly applies to the clinics where this reasoning confronts the uncontrollable nature of human biology. Most importantly, Sir Francis Bacon believed that hypothesis testing is only a final component of a process which included observation, experimentation and, finally, hypothesis testing. Thus, while deductive reasoning may represent a reasonable tool to move from the known to the unknown assessing one variable at the time, is not meant to enhance our general knowledge which can be easily achieved through direct observation⁽²¹⁾. With a human genome encompassing thousands of genes it seems logical to apply other reasoning strategies to extend our basic knowledge and identify hypotheses worthier of testing. Two strategies have been implemented to address this need: 1) first the identification of high throughput tools that allow the study of several hypotheses at once through the integration of available information into modular units and the development of prediction; 2) the implementation of discovery-driven inductive approaches aimed at the expansion of our knowledge of relevant facts with the purpose of crafting better hypotheses.

The high throughput approach can be applied to deductive or inductive reasoning. Following a top-down approach it is still possible to move from known to the unknown using more efficient bioinformatics tools; this approach is called “system biology” and, for the purposes of this perspective, we will refer to as “system immunology”^(90,91,92,93,94,95). Thus, instead of studying a gene or protein considered to be responsible for a given phenotype through a direct cause-effect relationship, system immunology integrates information from many genes and their products and constructs virtual networks similarly to mathematical models used by theoretical physicists to predict the distribution and behavior of matter throughout the Universe⁽⁹⁶⁾. However, since genes and proteins interact and modulate each other's expression through an infinite number of permutations, the linearity expected by deductive reasoning is rarely unambiguous and the resulting models become exponentially speculative as the process proceeds further. Thus, system immunology alone is not as efficient as mathematical modeling of the Universe where pattern recognition and computational tools combined allowed Einstein to predict the existence of invisible (dark) matter through the formulation of the cosmological constant theory and subsequently Vera Rubin to prove him right⁽⁹⁷⁾. This difference between the two disciplines is dictated by the fact that the rules governing the cosmos are much simpler and overpowered in the great distances by one force (gravity) over the others, while the biological micro-cosmos is affected by all biophysical

forces including gravity, electromagnetic and weak forces applied to each other at very short distances; thus as cleverly anticipated by Niels Bohr discussing physical principles, “prediction are difficult, particularly about the future”; in theoretical biology, it appears that predictions are difficult also when applied to the present.

Even Einstein's predictions and Rubin's validation were primarily based on observation or what is generally termed inductive reasoning. Importantly, inductive (or observational) reasoning does not require hypotheses as its primary goal is to sort facts that are relevant to a specific occurrence from those that are not. However, when in complex systems facts may become difficult to describe, inductive reasoning relies on non-linear mathematics (chaos theory) and the identification of general patterns that result from the sum of converging or diverging vectors directly or indirectly bearing a cause/effect relationship to the studied phenomenon^(83,84,85,86,87,74). Contrary to the child that observes individual clouds, the meteorologist will rely on repeated global hearth views from satellites and their value in weather forecasting and the clinical investigator on the application of high-throughput technologies to the analysis of human material in natural conditions or during the kinetic phases of disease⁽⁸⁶⁾. Indeed, “biology manifests several characteristics of chaotic systems” in which repetitions, given a sufficient number of permutations, progressively exfoliate random associations leaving a bare stem of recurrent patterns linked by necessity to a particular phenomenon. Identification of these recurrent themes segregates relevant from irrelevant observations⁽²²⁾. Thus, as an alternative to deductive reasoning, inductive reasoning aims at the identification of facts through observation and to reach broader generalizations based on the recognition that some facts are more closely linked to a particular phenomenon and, therefore, may be either directly or indirectly associated with its cause. Recently, Chaussabel et al.^(98,22) applied “bottom up” inductive reasoning to the direct study of diseases whose immunologic causation was either known or unknown. Based on the hypothesis that the transcription of peripheral blood monocytes (PBMCs) may be disease-specific, the authors analyzed PBMCs from individuals with different immune conditions and observed that distinct clinical entities displayed characteristic transcriptional patterns in which genes belonging to each pattern shared similar functions. Thus each cluster represented a transcriptional unit that could in about half of the cases be assigned a functional interpretation similar to the general biological principle of operons but conceptually broader. An example of functional unit are interferon stimulated genes (ISGs), whose expression, as discussed later, seem to be closely linked to several chronic and/or acute inflammatory processes⁽⁹⁹⁾. Interestingly, Chaussabel et al.⁽⁹⁸⁾ identified functional units whose expression was disease-specific and, in some cases, correlated with disease activity. Thus, following a bottom up approach, these authors provided an example of how system immunology could be approached through direct human observation creating functional entities that are based on facts rather than hypothesized biological interactions. Although the bottom-up, inductive strategy and classic system immunology top-down strategies are conceptually different, both aim at reducing the complexity and multidimensional information into a few, integrated, functionally-defined operative units that are more easily grasped by the human mind unprepared to process an excessive number of data points^(100,92).

A global approach to the study of humans and their diseases

It should also be emphasized that a global approach to the study of humans does not involve only high-throughput technologies and bioinformatics but, most importantly, the collection of human samples obtained at the right time and from the relevant tissues⁽⁸⁸⁾. In addition, several biological materials should be collected as each provides a different type of information. In an oversimplified view, humans, as multi-cellular organisms, are structured according to a hierarchy of interactions that go from genomic DNA, to transcription into

RNA and translation into functional units (proteins in different functional statuses) that may or may not differ among cells within a tissue or from different tissues. The study of each layer within this hierarchy provides distinct information: DNA analysis provides information about relatively stable characteristic of cells and tissues that may explain variations among individual patients, or aberrances between normal and abnormal tissues; messenger RNA (mRNA) informs mostly about the reaction of cells to environmental conditions; we compare transcriptional analysis to the electroencephalographic responses to stimulation which inform about the reaction to stimulus; thus, while mRNA provides information about the “brain response” of a cell (spikes in response to light), protein analysis provides information about what a cell is actually doing as the hand covers the eye when the light is too strong. Since each component provides different types of information and one kind cannot be assumed from the other, clinical research should study humans by evaluating all components simultaneously at moments relevant to the natural history of a disease or its response to therapy. Although this global approach may appear burdensome, it is, in our opinion, the most effective way of addressing human pathology rather than depending on correlative studies of large populations in which few parameters are studied without knowledge about potential modifiers⁽⁸⁸⁾.

Of common immunologic themes and the immunologic constant of rejection

Chaotic systems are not necessarily impervious or even unfriendly to scientific speculation. In fact, by continuously generating a large number of permutations provide an infinite number of irrelevant information that can be easily sorted from the relevant data by necessity associated to a particular occurrence. As discussed in the previous section, Chaussabel et al.⁽⁹⁸⁾ reported functional units that could distinguish individual disease patterns and, in some cases, prognosticate their clinical spectrum. This information will likely be useful for diagnostic and prognostic purposes. We, however, propose an alternative strategy aimed at the identification of common patterns that lead to similar outcomes independently of the originating cause; in immunology, relevant and recurring phenomena are immune-mediated tissue-specific destruction (TSD) and tolerance. Although infections, autoimmunity, allograft rejection and cancer are dealt differently in a disease-specific manner by the host's immune response, it is logical to hypothesize that tolerance or destruction of a given tissue may follow in the end common mechanisms. Thus, as done in comparative immunology, clinical scientists should consider adopting the classic principle that recurrent themes are relevant to a specific determinism and attempt to identify them through direct human observation^(99,101).

We recently published a commentary describing a rudimentary and preliminary yet, hopefully, thought-provoking example of the potential of comparative immunology^(3,99). As previously discussed, the relationship between cancer and its host is complex and multifactorial. Yet, “it is a mistake to imagine that complex diseases may not be solved by simple approaches”⁽¹⁰²⁾. In 1969, Jonas Salk observed that 1) delayed hypersensitivity reaction to tuberculin; 2) contact dermatitis; 3) graft rejection; 4) tumor rejection; and 5) autoimmune phenomena represent facets of a similar immune-mediated phenomenon that he termed “*delayed allergy reaction*”⁽¹⁰³⁾. Following Salk's intuition, we supposed that TSD that spares the rest of the host's tissues represents a singular entity. TSD can be triggered by distinct mechanisms during allograft or tumor rejection, autoimmunity or pathogen clearance; however, the effector mechanisms converge into an identical final pathway: the immunologic constant of rejection (ICR)⁽⁹⁹⁾. The ICR hypothesis is based on four axioms: (i) Tissue-specific destruction does not necessarily occur because of non-self-recognition but also occurs against self or quasi-self. (ii) The requirements for the induction of a cognate immune response differ from those necessary for the activation of an effector one. (iii)

Although the prompts leading to tissue-specific destruction vary in distinct pathologic states, the effector immune response converges into a single mechanism that includes the activation of adaptive and innate cytotoxic mechanisms. (iv) Adaptive immunity participates as a tissue-specific trigger, but it is not always sufficient or necessary for tissue destruction⁽⁹⁹⁾.

Characterization of the common immunologic pathway leading to TSD may suggest simplified strategies for the treatment of immune-mediated conditions; by regulating its critical component it may be possible to avoid tissue damage in the case of autoimmunity or allograft rejection or, conversely, induce the beneficial elimination of disease-affected tissues such as cancer or virally-infected cells⁽²¹⁾. With this approach, we first identified recurrent themes in cancer tissues undergoing destruction in response to IL-2 therapy⁽¹⁰⁴⁾, active-specific immunization⁽⁶¹⁾ or the TLR-7 agonist Imiquimod⁽¹⁰⁵⁾ and compared common patterns with signatures associated with good prognosis in patients with primary colorectal⁽¹⁰⁶⁾ or ovarian carcinoma⁽¹⁰⁷⁾, allograft rejection⁽¹⁰⁸⁾, successful treatment of hepatitis C virus (HCV) infected patients with IFN- α ⁽¹⁰⁹⁾ and clearance of HCV during acute infection⁽¹¹⁰⁾. By comparing ours with others' observations, we realized that TSD follows a common cascade centered on activation of IFN- γ dependent genes such as the CXCL-9 through -11 chemokines, combined with the expression of CXCL12 and CCL-5 chemokines (CXCR3, CXCR4 and CCR5 ligands respectively). These in turn, recruit large numbers of activated T and NK cells, which express genes encompassing several immune effector functions (IEF) including granzyme-B, perforin and FAS. Thus, the immunologic constant of rejection depends on the expression of ISGs related to type II IFN (such as IRF-1), IFN- γ -modulated chemokines and a cluster of IEFs⁽⁹⁹⁾.

Following a bedside-to-bench approach^(20,80,111), we then tested the ICR hypothesis in mice. GLV-1h68 is an attenuated recombinant vaccinia virus (VACV) construct with therapeutic and diagnostic properties that colonizes selectively established human xenografts and induces their complete regression. We previously documented that the regression of the xenografts is at least in part immunologically mediated through the activation of ISGs and innate IEF mechanisms⁽¹¹²⁾. In a follow up study (*Worschech et al. manuscript submitted*), we explored human cancer cell/VACV interactions *in vitro* and xenograft/VACV/host interactions *in vivo* adopting organism-specific expression arrays. Tumor rejection was associated *in vivo* with activation of innate immune effector mechanisms correlating with VACV colonization of the xenografts. Most importantly, *in vitro* signatures were identified in uninfected cultured cancer cell lines predictive of delayed GLV-1h68 replication *in vitro* and lack of *in vivo* responsiveness of the respective xenograft. GLV-1h68 is being evaluated in phase I clinical studies as a systemic anti-cancer treatment. Thus, we propose to include these signatures in clinical settings to evaluate their predictive value as biomarkers of tumor responsiveness and help the interpretation of the clinical results. Most of these genes have been described by us as constantly expressed during TSD according to the immunologic constant of rejection hypothesis confirming that the pathway is consistently observed also in animal models and supporting the concept that immune-mediated rejection can occur in immune deficient mice in the absence of adaptive immune responses⁽⁹⁹⁾. The relevance of interferon stimulated genes is become increasingly apparent and central for several diseases spanning from autoimmunity⁽¹¹³⁾ to virally-induced degenerative diseases⁽¹¹⁴⁾. In all cases, their presence is clearly necessary although it remains to be ascertained what other factors are intrinsically necessary for disease induction and/or resolution. As these recurrent pathways leading to immune-mediated tissue damage will become increasingly uncovered, a more definitive and specific road-map will hopefully be constructed that could lead to the definition of a more rational approach to the immunotherapy of multiple diseases.

Future perspectives in biomarker discovery

We offered a novel and not exclusive perspective to the study of human pathology. As predicted by Jonas Salk⁽¹⁰³⁾ Nature follows a relatively conserved path that is independent of the disease model and can be easily observed across disciplines. Several initiatives are following the principles described here such as the International Society of Biological Therapy for Cancer (iSBTc) attempt to identify common biomarkers predictive of treatment outcome⁽⁸⁸⁾. A similar goal has been proposed in Europe by the Cancer Immunotherapy Monitoring Program, in the United States by the Immune Tolerance Network⁽¹¹⁵⁾; A new Federation was developed dedicated to interdisciplinary exchanges in clinical immunology: the Federation of Clinical Immunology Societies⁽¹¹⁶⁾. Importantly, several institutions are recognizing similar needs. Stanford has initiated a facility aimed at the global and centralized monitoring of human immunology⁽¹⁵⁾. At Baylor (Dallas, TX) a center for integrative basic and clinical immunology has been developed that has provided already promising results following an inductive approach^(113,98,117). Finally, the NIH intramural program initiated this year a trans-institutional program aimed at the study of common aspects of immunology in genetic and functional terms across disciplines; this Center for Human Immunology (CHI) integrates high-throughput phenotyping of immune cells⁽¹¹⁸⁾ with genetic^(119,48,47), system immunology^(120,121,122,92,95) and observational approaches^(123,61,105,68,99,22). The challenges to the accomplishment of this integrative approach are formidable including the extensive bioinformatics support required. They have been recently summarized by the iSBTc discussing the goals of a task force organized together with the United States Food and Drug administration⁽⁸⁸⁾. The task force has the purpose of taking a programmatic look at innovative venues for the identification of relevant parameters to assist clinical and basic scientists who study the natural course of host/tumor interactions or their response to immune manipulation. The task force has two primary goals: 1) identify best practices of standardized and validated immune monitoring procedures and assays to promote inter-trial comparisons and 2) develop strategies for the identification of novel biomarkers that may enhance our understating of principles governing human cancer immune biology and, consequently, implement their clinical application. We firmly believe that these and similar initiatives will need to be implemented and supported in translational setting to achieve effective progress in the treatment of immune-mediated pathologies.

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Executive Summary

- Experimental models are not always representative of human disease and its response to treatment
- Humans, contrary to inbred experimental systems, are genetically diverse and most of their diseases are heterogeneous
- The complexity of human biology can only be encompassed by non-linear mathematical models
- Hypotheses relevant to human disease can and should be generated through an inductive approach
- Strategies to effectively study with inductive methods human pathology at a global level
- Potential for identifying common themes that are necessary for the occurrence of a given phenomenology and could, as a consequence, represent universal targets of therapy