

Cell mediated immunity to meet the avian influenza A (H5N1) challenge

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Abbreviations

ARDS: Acute Respiratory Distress Syndrome; CBO Congressional Budget Office; CMI: Cell-Mediated Immunity; CMV: Cytomegalovirus; EBV: Epstein Barr Virus; HHV-6: Human Herpes Virus 6; HSV: Herpes Simplex Virus; LMI: Leukocyte Migration Inhibition; SIV: Simian Immunodeficiency Virus; TF: Transfer Factor; VZV: Varicella Zoster Virus.

Abstract

Avian influenza A subtype H5N1 virus with its recombination potential with the human influenza viruses presents a threat of producing a pandemic. The consensus is that the occurrence of such a pandemic is only a matter of time. This is of great concern, since no effective vaccine is available or can be made before the occurrence of the event. We present arguments for the use of cell mediated immunity for the prevention of the infection as well as for the treatment of infected patients.

Transfer factor (TF), an immunomodulator of low molecular weight capable of transferring antigen-specific cell mediated immune information to T-lymphocytes, has been used successfully over the past quarter of a century for treating viral, parasitic, and fungal infections, as well as immunodeficiencies, neoplasias, allergies and autoimmune diseases. Moreover, several observations suggest that it can be utilised for prevention, transferring immunity prior to infection. Because it is derived from lymphocytes of immune donors, it has the potential to answer the challenge of unknown or ill-defined pathogens. Indeed, it is possible to obtain an antigen-specific TF preparation to a new pathogen before its identification. Thus, a specific TF to a new influenza virus can be made swiftly and used for prevention as well as for the treatment of infected patients.

Introduction

An avian influenza A virus (H5N1) has recently appeared in Asia (1, 2). It has crossed the species barrier infecting patients, and often lethally so (3-5). Its pathogenicity is high and increasing (6-8). Usually a respiratory failure is caused by diffuse, ground-glass infiltrates and manifestations of the acute respiratory distress syndrome or ARDS. Its potential of generating a pandemic is extremely high, especially by recombination in infected patients with the seasonal human influenza virus rendering the new viral entity highly contagious, and making human to human transmission possible.

The strategy to avert this threat is use of antivirals for treatment, and vaccination for prevention. Regrettably, the number of effective antivirals available is limited, and the preparation of a vaccine at the experimental stage. However, another approach is worth considering: the use of cell mediated immunity (CMI), viz. an influenza-virus-specific Transfer Factor (TF) for the prevention and the treatment of the infection.

The concept of TF was first reported in 1954 (9-11). Over fifteen [hundred publications](#) have since established that lymphocyte-dialyzable-extracts from immune donors can transfer antigen-specific CMI information in vitro to naive lymphocytes or in vivo to patients and experimental animals. Since CMI plays a crucial role in the control of infectious, parasitic, autoimmune diseases, and cancer, TF has been used over the past thirty years for treating such conditions and sometimes with dramatic success. Because it was believed to be species-specific, for many years TF was prepared from the lymphocytes of immune blood donors, viz. patients household contacts. In 1974, it was shown that human TF with known specificities could be replicated in tissue culture, using a lymphoblastoid cell line (12,13), and in the late 1970's, evidence was presented that antigen-specific TF obtained from mammals after immunisation with a given antigen was active in humans (14,15).

If an active-household TF can be used for patients treatment, or replicated in tissue culture, it is obvious that the immune cells recognize epitopes of interest, even if the physician has not identified the pathogen. This '*black box*' effect can also be used for TF production in animals, i.e. by injecting laboratory animals with a TF from household contacts, thus solving the availability problem especially for non identified pathogens. Moreover, animal immunisation with partially identified microorganisms may also be utilised for production of a TF with a new specificity.

The rationale for the in vitro replication stems from the original Lawrence observations. He had shown that a TF of the donor's specificity can be retrieved from a naive recipient whose lymphocytes apparently act as a photocopier multiplying the injected TF and thus becoming a subsequent effective donor. Biochemical data suggest that the activity is carried by a ribonucleopeptide of ca 5000 Da. Yet, at the molecular level, the mechanism of action remains largely unknown, and because of the presence of a blocked amino terminus, attempts to sequence the peptide have failed. Recent work has partially identified the amino-acid sequence, thus giving partial biochemical identity to a still elusive moiety (16). However, in order to grasp the mode of action, unravelling the rest of the peptide sequence and identifying the ribonucleotides remains of the essence.

Although the transfer of antigen-specific CMI information by this extract is thought-provoking and has been challenged on theoretical grounds, the experimental evidence, albeit readily reproducible has

never been contested (17). Various explanations for understanding the mechanism have been proposed, but so far none has been proven entirely satisfactory. For instance, studies with such rare antigens as coccidioidin (18) or keyhole limpet haemocyanin (KLH) (19) preclude non-specific enhancement of lapsed pre-existing immunological memory.

At least three types of antigen-specific activities are present in lymphocyte dialysates: inducer or helper, suppressor and cytotoxic [20]. Data suggest that antigen-specific inducer and antigen-specific suppressor and cytotoxic transfer factors may be derived respectively from CD4 and CD8 lymphocyte sub-populations (Viza, unpublished data). This accounts for the immunomodulating activity of the extract, boosting the immune response via the CD4 lymphocytes, and decreasing the immune over-reactivity by stimulating the suppressor cells.

TF has been proven to be an effective treatment for a variety of pathologies. In favour of its clinical use, one should stress its lack of toxicity and the absence of side effects. Indeed, for over three decades, several hundreds of patients have received large amounts of TF and none has ever reported signs of acute or chronic toxicity when TF was prepared by expert physicians.

Clinical observations

Since the 70's seminal work of Fudenberg's group on the use of TF for treating neoplasias, viz. osteosarcoma (21-23), numerous publications have produced evidence that TF can be used as an adjuvant treatment for cancer, e.g. Burkitt's lymphoma (24), nasopharyngeal carcinoma (25), urological neoplasias (26,27). Several parasitic diseases are known to respond to TF therapy, e.g. cutaneous leishmaniasis (28-30), schistosomiasis and cryptosporidiosis (31), whereas rare syndromes such as Behçet's, probably of viral origin, and Wiskott-Aldrich's genetic immunodeficiency are both responsive, and sometimes spectacularly so to this treatment (32-35). Other studies have shown that antigen-specific TF may produce a spectacular improvement in acute cytomegalovirus (CMV) infections (36), whereas African children suffering from Burkitt's lymphoma – a tumour caused by the Epstein-Barr virus (EBV) in Africa – treated over a long period with EBV-TF showed a significant decrease in the rate of relapses (24). Similar noteworthy results were obtained in Malaysia in preventing relapses of nasopharyngeal carcinoma – caused by EBV in South-East Asia – by the administration of an EBV-specific TF (25). HHV-6 infections, often the cause of the chronic fatigue syndrome, seem also to respond to HHV-6-specific TF (37).

TF preparations have been utilized for preventing viral infections. In a controlled clinical trial, [Steele and co-workers](#) were able to protect leukaemic children receiving chemotherapy from varicella zoster virus (VZV) infections using a VZV-specific TF (38, 39). These observations confirm the efficacy of TF in fighting viral infections, and introduce the concept of prevention. In the 1980's, several investigators described significant improvement by the use of HSV-specific TF from human or bovine origin in treating patients suffering from recurrent genital and/or labial herpes (40-43) or recurrent herpes keratitis (44, 45). These clinical observations were corroborated by data in a mouse model: HSV-specific TF was able to prevent mouse mortality following a lethal injection of the HSV virus [46], thus confirming the preventative potential of this extract, already suggested by Steele and co-workers

in 1976 (47) : TF from a HSV-1 patient protected marmosets from a lethal HSV-1 challenge. It thus appears that all three classes of herpes viruses i.e., alpha (HSV, VZV), beta (CMV, HHV-6), and gamma (EBV) respond to specific-TF treatment. Furthermore, preliminary studies with SIV-specific TF have produced significant results in macaques consequently challenged with SIV (48), whereas chronic active hepatitis B patients considerably improve when treated with Hbsag-specific TF (49, 50). It is worth mentioning that TF derived from mouse CD8 lymphocytes immunised with pollen and house dust has shown an inhibitory effect in vitro in the LMI test (51), and has significantly improved patients suffering from asthma or seasonal hay fever (Viza and Hebbrecht, unpublished observations).

An avian-influenza-virus-specific TF

The present bird epidemic due to the influenza A subtype H5N1 virus and its mutation and recombination potential with the seasonal human influenza viruses creates a risk of a human pandemic (52-54). Indeed, the spreading of the infection seems unavoidable, the virus has been found not only in domestic poultry and migratory birds, but also in birds smuggled across borders (55), and its recombination with the human virus is only a matter of time. Observations that birds can be healthy carriers of the virus several weeks before any signs of the disease increase the odds of a world wide spread infection of poultry, domestic animals and eventually humans. The recent appearance of the virus on the African continent confirms its relentless progression.

Against such a pandemic and with only one main antiviral at hand, it is difficult to imagine an effective containment of the infection, the emergence of resistant strains being inescapable. The Congressional Budget Office (CBO) estimated that *"a severe pandemic of avian flu could hit the United States hard, killing 2 million Americans and pushing the economy into a major recession"*. The agency underlined that such pandemics are unpredictable, and looking at episodes back to 1700, the odds of an influenza pandemic in any given year are about 3% to 4%. There were three global pandemics in the 20th century (56).

Past work (57) suggests that human influenza-virus-specific TF can be prepared by laboratory animal immunisation. Its activity was tested in vitro in the LMI test as well as in vivo by transferring CMI across the species barrier into mice. It was used for protecting rodents against a virus challenge, thus suggesting that an influenza-specific TF can confer protection against this virus. Furthermore, it is worth mentioning that despite the variability of the seasonal influenza virus due to its high mutation rate, it has been observed that patients receiving TF treatment for various clinical conditions e.g. herpes, candidiasis or cancer, suffered less frequently from seasonal flu (Pizza and Viza: unpublished independent observations). It is plausible that a TF prepared from the lymphocytes of a pool of blood donors may carry antigenic information from influenza virus strains that have infected the donors, and that some antigenic determinants are shared by the *'year's strain'* thus conferring the observed protection.

Every avenue should be explored for preventing the foreseen and seemingly unavoidable pandemic (8). By inference from past observations, it is plausible to hypothesise that a TF made against a new emerging influenza virus will be effective for curtailing its spreading if used as a vaccine. Such a TF

may be prepared by animal immunisation with the new virus when available. But it is also plausible to assume that a TF made from animals injected with the H5N1 subtype, before any recombination, could be at least partially effective, since the recombinant virus would be sharing antigenic determinants with the H5N1 subtype. It could thus confer some CMI protection. Epitopes responsible for generating an efficient CMI response are not necessarily identical to those responsible for a neutralising antibody response.

Vaccines

Existing and to-be-developed influenza antivirals have two potential drawbacks: toxicity and development of viral resistance. Viruses, especially those with high mutability, always produce resistant strains to existing antivirals. Moreover, all antivirals present short or long term toxicity, producing, inter alia, kidney failure. Expertly prepared TF is totally innocuous, and so far no viral resistance has ever been reported. And even if such a resistance were to appear, it would be easy to produce a new generation TF in order to counter it. This option should be seriously considered as we don't seem to be ready to face such a pandemic at present: "*vaccines are the mainstay of prophylaxis, but there are technical and safety issues that must be overcome in the development of vaccines in order to combat avian influenza*" (58). A virus-specific TF can be a substitute for such vaccines.

If the ideal solution to the pandemic challenge would be world wide vaccination with a potent vaccine, such a vaccine is not available at present. Early vaccines were poorly immunogenic [59], and required the addition of adjuvants to produce an antibody response [60, 61]. Hence the consensus that further studies with approved adjuvants, e.g. alum, are needed. However, because of their adjuvants, viz. alum, vaccines may produce serious side effects, as those documented in France from the use of hepatitis B vaccine (e.g. several cases of multiple sclerosis and other neurological and autoimmune disorders), and those observed in the Gulf War syndrome. Recent attempts to prepare an egg-independent vaccine using replication-incompetent human adenoviral-vectors have been encouraging (62, 63), but all potential hazards from their wide spread use in human populations have not been investigated. The failure for the last twenty years to produce an effective AIDS vaccine, and certain dramatic adverse side effects observed when viral vectors were used to correct genetic defects, call for extreme caution at this stage.

Down regulation of inflammatory reactions

The rationale for using TF for the treatment of avian influenza A infection is two fold: boosting specifically the immune defences against the virus, and down regulate the inflammatory reaction. Indeed, elevated blood levels of interleukin-6, TNF- α , interferon- γ , and soluble interleukin-2 receptor were observed in individual patients [64] and human macrophages exposed to the virus show increased cytokine production, viz. TNF- α [65]. Other studies showed elevated levels of chemokines, interferon-inducible protein 10, monocyte chemoattractant protein 1, and monokine induced by interferon- γ , three to eight days after the onset of illness [66]. Such responses are probably at least partially responsible for the ARDS and multiple organ failure observed in many patients. It thus appears that the immune reaction to influenza A (H5N1) virus contributes to disease pathogenesis and

mortality. Plasma levels of inflammatory mediators (interleukin-6, interleukin-8, interleukin-1b, and monocyte chemoattractant protein were found to be higher among patients who died than among those who survived [Simmons C: personal communication, cited in the WHO report (67)], whereas the average levels of plasma interferon- α were about three times as high in patients with avian influenza A who died as among controls (67).

To combat the H5N1 virus induced inflammatory syndrome, immunomodulators such as corticosteroids have been rationally proposed, but with disappointing results. In five patients given corticosteroids in 1997 two survived, whereas in Vietnam, all four patients given dexamethasone died (67). To this end, other immunomodulators such as interferon- α , which possesses both antiviral and immunomodulatory activities have been proposed for controlled clinical trials. TF should be able to down-regulate such disorders. For instance, it has already been reported that it has a regulating in vitro and in vivo effect on lymphokines such as IL-4, IL-6, TNF- α , and IFN- γ (68-70 and Viza and Pizza, unpublished data).

Discussion

Exposure to infected poultry is widespread, but so far the species barrier seems to confer significant protection to the avian H5N1 strain. Nevertheless, this barrier is not insurmountable as the infection of several patients and other mammals has proven (3-5). Besides, such viruses evolve. By changing their gene arrangement and antigenicity (67, 71-74) they will eventually become more human adaptable, and reports already confirm that the H5N1 now displays increased pathogenicity in laboratory mammals (6-8).

The use of CMI and TF for prevention is an option that should be carefully considered. It concerns not only the present threat of the H5N1, but also the 'new' viruses of the past such as SARS or EBOLA, and the 'new' viruses of the future. Several reports suggest that when a virus-specific TF is administered before the encounter with the virus, the recipient is protected. The most significant studies in this respect are those of Steele et al. (38, 39, 47) and Viza et al. (46). Furthermore, a virus-specific TF can be used for treating patients [e.g. 40, 41]. As with antivirals, early TF administration should provide the greatest clinical benefit to infected patients.

Although faster than a mass production of a conventional vaccine, TF's manufacture utilising an attenuated strain of the recombinant virus for animal immunisation, once the new pathogenic strain has been isolated, is still a lengthy process and requires a sufficient stock of the attenuated strain. An alternative method of production could be the injection of animals with TF obtained from infected patients or from animals immunised for this purpose with the first viral cultures of the new virus. Such a TF may be replicated by injection into naive animals. Lawrence's work has shown that a TF carrying the initial specificity can be retrieved at the end of at least 5 serial injections from donor to naïve recipient, each recipient replicating the injected TF and serving as donor for the next recipient. By applying an analogous procedure, an effective recombinant-H5N1 TF could be produced without delay. Large animals such as calves or pigs (5) could be used for the initial immunisations.

The two procedures for producing a virus-specific TF i.e., in vitro replication and animal immunisation are efficient, expedient and cost effective. Indeed, cost as always is an important factor to consider. A recent joint WHO/UNICEF study points out that for the vaccination of less than 70% of children in 72 of the poorest countries, the annual budget is 2.5 billion dollars. Despite the massive contribution of Bill and Melinda Gates' Foundation, one additional billion dollars per annum is needed to immunise up to 90% of the children's population. But only one third of the budget serves for purchasing the vaccines, refrigeration and storage representing over the third of the remaining cost. TF in its present freeze-dried form for oral administration makes storage and transportation easy and inexpensive. Indeed, although at the early days TF was administered by subcutaneous injections, it was shown that oral administration is as effective [e.g. 41-45]. The amount of TF extract needed for treating patients orally is less than 1 mg per day, the same amount that was used at the time of TF injections and for animal experiments. Five to 6 mg over a ten day period should be sufficient to confer significant protection. Moreover, the efficacy of the nasal route seems plausible and should be investigated.

Despite controversy generated by TF's characteristics (low molecular weight, undefined chemical structure, unconventional mode of action, resistance to most proteolytic enzymes) and biological properties (non-species specificity, transfer of antigen-specific information), its clinical applications have benefited several hundred patients [e.g.16]. An impressive number of clinical studies have proven its efficacy in treating and preventing infections due to viruses and other microorganisms. Its potential against new emerging pathogens that could have dramatic consequences to human health should be investigated. Moreover, if the benefits of conventional vaccines may be often offset by extremely serious side effects, utilising TF as a prophylactic vaccine addressing CMI is of paramount interest. The avian influenza A epidemic provides an opportunity for studying and exploiting this potential in order to curtail the spreading of the virus and avert the pandemic.

Albeit innocuous and effective when skilfully prepared, TF may be totally ineffective when made without expertise. And since it is practically impossible to assess its potency in vitro or in animal models outside an adequately equipped laboratory, any unscrupulous manufacturer could exploit the placebo effect of multicoloured capsules. Caution is therefore of the essence when purchasing commercial preparations today readily available and offered as food supplements via the web.

Conclusion

Extrapolating from past observations, we argue that cell mediated immunity can be used for the treatment and the prevention of viral infections. The avian influenza virus (viz. the strain H5N1) with its recombination potential with the human influenza virus presents the risk of a pandemic. As no vaccine can be made until the emergence of the new virus, we propose an alternative approach: the use of a transfer factor to the new virus to enhance cell mediated immunity. Its use as a preventative vaccine based on cell mediated immunity holds great promise in the fight of viral infections, even prior to the identification of emerging microorganisms. Because it can be made swiftly and be used as a preventative vaccine as well as for the treatment of infected patients, such a preparation may be an effective response to this viral challenge.

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