Autoimmune T-cells induced by low dose immune checkpoint blockade could be a powerful therapeutic tool in cancer through activation of eliminative inflammation and immunity

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"*It is not the strongest of the species that survives, nor the most intelligent, but the one most responsive to change*." Charles Darwin

Summary

The immune system has been hypothesized to have evolved to purge nascent selfish host cells, while immune defense against xenogeneic alien pathogens appeared later in evolution. To prevent a natural tendency of tumor development, we proposed that immune surveillance is carried out by a coupled system of complementary T cells and host cells. An ongoing internal dialogue between T cells and host cells keeps T cells alive via stimulation by self-antigens while putting strict limits on variations of host cells by eliminating selfish cells. Convincing evidence for such dialogue, which temporarily activates T cells, emerged from the widespread autoimmune events in 72% of advanced melanoma patients treated with the immune checkpoint blocking ipilimumab. This blockade turned physiologic T cell activation into uncontrolled autoimmunity. We suggest that harnessing the unleashed autoimmune power of T cells would be more rewarding to eliminate cancer than copying infectious vaccination to induce tumor specific immunity.

Keywords: cancer deaths; infectious disease deaths; one-signal T cell model; ipilimumab; harnessing autoimmune T cells; fallacy of cancer immune therapy.

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1. Introduction

Alfred Tauber proposed that "…'immunity' may be a semantic trap that has confined our understanding of the immune system to only a narrow segment of defensive, aggressive functions" [1]. Satisfactory answers have not been available to explain 'why invertebrates including more than two million species in more than 20 phyla use only germline encoded innate immunity', or 'why vertebrates reject any allogeneic or xenogeneic transplanted tissue'. Baruch Rinkevich has challenged the tacit assumptions and dogma that evolution of the immune system is pathogenically directed. Instead, he proposed that immunity developed as a surveillance operation to purge nascent selfish cells that littered the soma and the germline. Accordingly, the primary role of the vertebrate immune defense is to preserve individual integration. From this point of view, defense against pathogens, which are xenogeneic aliens, would have appeared later in evolution [2].

Textbooks portray the immune system following Niels Jerne's view that it stands in readiness to deal with the entire antigenic universe. Such idealization neglects several important problems at the expense of biological common sense [3]. Based on information theoretical principles and the law of parsimony, we proposed first that for surveillance T lymphocytes should recognize the much smaller set of self-antigens, rather than the practically unlimited non-self-antigen universe. To this end, we developed a new model in which individual integrity can be preserved from parasitism with a limited T cell repertoire, where one-signal is sufficient for activation [4] [3] [5]. This is achieved by a homeostatic coupled system based upon an internal dialogue between the positively selected, low affinity complementary T cells and host cells. In our model, the role of regulatory T cells (Foxp3+ Tregs) seems to be the closest analogy to the role of homeostatic T cells (our one-signal model was compared with well-established concepts in great details earlier in [3]). It is reassuring that close association and communication between Treg cells and plasma cells was indeed demonstrated recently in the bone marrow [6]. This suggested that Treg cells affect the homeostasis of many cell types through cell contact or via soluble factors.

Vindication for an ongoing internal dialogue between complementary T cells and host cells has emerged from an unexpected origin: widespread autoimmune adverse events in advanced melanoma patients receiving the checkpoint blocking anti-CTLA-4 antibody (ipilimumab). A meta-analysis of ipilimumab mediated autoimmune-related adverse events (irAEs) in 1265 patients from 22 clinical trials included in the pooled analyses found an incidence of 72 % for all-grades of irAEs and 24 % for high-grade irAEs, leading to hospitalization or intravenous treatment of patients. The incidence of all-grade irAEs varied according to the dosage of the drug, from 61 % in patients receiving ipilimumab at 3 mg/kg to 79 % in patients treated with 10 mg/kg. These observations corroborate the idea of coupling autoimmunity and tumor immunity as argued below [7].

2. Ipilimumab clinical trials – An alternative interpretation of severe, widespread autoimmune-related adverse events

Although the CTLA-4 blockade is persistently thought to be tumor specific, clinical remission (partial or complete), or at least cancer stabilization, was noted for the majority of patients who experienced autoimmune-related adverse events (irAEs). In our view, the widespread and dose-dependent irAEs can be better explained by our one-signal model [8] [9] [10] [11] than a mechanism based on the classic "two-signal" model of T cell activation as described briefly below.

The consensus view holds that to maintain tolerance, T cell antigen receptor (TCR) input must be complemented by CD28 co-stimulation to promote interleukin-2 (IL-2)–dependent proliferation, as described by the classic "two-signal" model. This is taken to mean that each cell requires

the conjoint signals for these two receptors triggering activation to a state suitable for cell division (see in [3]).

However, according to the law of independent T cell activation, there is no need for an obligatory co-stimulus. The internal mechanisms that control the rate of division, the likelihood of surviving, and the likelihood of undergoing a differentiation operate independently within a cell. In fact, the strength of a T cell response can be predicted by adding together the underlying signal components from the TCR, co-stimulatory receptors, and cytokines. This law resolved the co-stimulation paradox and provided a quantitative paradigm for therapeutically manipulating the strength of the immune response [12 13].

Since immune cells require regular stimulation for survival we proposed that self-antigens, from time to time, activate T cells through the internal dialogue via one-signal mechanism. Temporarily activated T cells express CTLA-4, which is blocked by anti-CTLA-4 antibodies not only on tumor specific T cells but also on all activated T cells. Abrogation of the function of CTLA-4 permits CD28 to function unopposed, swinging the balance in favor of immune stimulation, tolerance breakdown, and eventually tumor eradication.

This proposal is consistent with the *homunculus* concept of autoimmunity theory developed by Irun Cohen, who has argued that the aim of the immune system is *not* to discriminate self from non-self, but continuously respond to self in order to organize inflammation in a way that heals and regenerates damaged cells and tissues. This is carried out with a high frequency by lymphocytes that recognize key self-antigens: the *immunological homunculus* [14]. Evolution may well provide an answer as to why such constant self-peptide control might be necessary.

3. Viruses playing a pivotal role in evolution very likely increase the risk of DNA damage and cancer

In the biosphere the viral genes outnumber cellular ones [15]. The delivery of genes from virus to invaded cell is far more overwhelming when compared with the reverse event, i.e. transfer of genes from host cells to viruses. It had been proposed that ancient viruses in evolution spontaneously acted as essential editors of the host genome [16]. Viruses then may be thought of as the molecular tools of "*Nature*'s genetic engineering laboratory" that manipulate key aspects of animal biology. Accordingly, such natural genetic engineering in evolution would have contributed to the emergence of evolutionary innovations. Genetic engineering mediated by viruses in nature, however, is a double-edged sword. Whereas viral gene transfer might have speeded up the evolution of the species, viral remnants, e.g. jumping genes, represented a real danger to the individual by increasing the risk of DNA damage, cancer and other pathological conditions. Genomes with various interactions are likely to be hotbeds of evolutionary conflict. [B]

The success of multicellularity depends upon the evolution of mechanisms that are able to suppress the ability of virtually every cell in an organism with the information and the potential to propagate rapidly. Protective mechanisms that evolved over millions of years are indeed capable of keeping the incidence of cancer very low (~2%) during reproductive age [17]. Epidemiological observations are consistent with the theory that immune protection against cancer appeared earlier in evolution than against pathogens and therefore it is more effective against selfish host cells than against xenogeneic aliens.

4. Age-related cancer incidence

Before modern medical achievements the likelihood of an individual dying prematurely from infectious diseases was as high as 40%. [C] In contrast to the infectious

[B] http://www.the-scientist.com/?articles.view/articleNo/42274/title/Wrangling-Retrotransposons/

[C] http://www.cdc.gov/mmwr/preview/mmwrhtml/mm4829a1.htm#fig1

death rate, only one-third of humans are struck by cancer, mainly with advancing age [18]. Fortuitously, good supporting historical evidence is available in the Statistical Yearbook of Hungary from 1896 about all causes of death and cause-specific mortality. [D] The data show that deaths due to infections were 27%, whereas deaths due to cancer were only 2%. It must be noted that the mortality rate of 27% from infectious diseases is a conservative estimate, since pneumonia, bronchitis, meningitis and encephalitis were not included in deaths due to infectious diseases. It is noteworthy that a similar difference between the mortality rate from infectious disease and other diseases was recorded in the USA. Cutler and Meara reported that at the beginning of the 20th century, deaths due to infections were 32%, whereas only 5% due to cancer. [E] In the low-income countries, where modern medical advances are not readily available, this ratio (28% vs. 6%) had not changed much by 2012. [F G H]

Most human malignant tumors remain latent for many years when detected by present-day diagnostic methods. It takes more than 30 doublings for the tumor cells to reach a population size of 10^9 cells. At that point the tumor has a diameter of 1 cm and weighs 0.52 g. Depending on the tumor volume doubling time, it is then 2 to 12 years old [19]. Consistent with this observation, the average age of women with pre-invasive lesions is about 20 years lower than for those with invasive lesions. [I] The risk of cancer increases exponentially with age [20]. The risk of breast cancer, for example, increases from 1 in 400 at thirty years of age to 1 in 9 at seventy years of age. Age incidence curves rise sharply above the age of 50 years and are informative about the dynamics of tumor progression [21]. Although two out of three humans never develop clinically detectable cancer [18], most individuals with no apparent pathology, but having died of trauma, at autopsies were discovered to have been harboring unsuspected microscopic primary cancers [22]. The risk of suffering any cancer before the age of 40 is ~2%, but by age 80 this risk increases to 50% [17].

In contrast to the growth profiles of tumors, the number of bacteria doubles in 20 min, whereas viruses produce more than 1,000 progenies in a few hours generating hundred times more virus infected cells within a few days than cancer cells develop during many years. A good example for this is hepatitis B virus (HBV) infection. According to Guidotti and Chisari [23], in the unlikely event of all the 10^8 HBV-specific CTL in the entire body entering the liver at the same time and all the 10^{11} hepatocytes quite commonly infected, for every 1,000 infected hepatocytes, there would be only one specific CTL in the liver to cope with the infection. Obviously, 1:1000 ratio would be totally inadequate for cytotoxic mechanism alone. Nevertheless, the immune system of most infected patients clears the virus within a few weeks without serious liver disease. This fact indicates the contribution of non-cytopathic mechanisms. We speculate that specific cytotoxic T lymphocytes (CTL) that emerged during evolution to cope with slowly growing cancer cells are unable to control the explosive expansion of virus-infected cells by themselves without additional mechanisms.

[D] http://kerepesi.web.elte.hu/causes_of_death/1896_leading_causes_of_death_en.txt-krona.html

[E] See Table 3 in http://www.nber.org/papers/w8556

[F] http://apps.who.int/gho/data/view.main.CODWBINCLOINCV?lang=en

[G] http://kerepesi.web.elte.hu/causes_of_death/low_income_leading_causes_of_death.txt-Krona.html

[H] http://kerepesi.web.elte.hu/causes_of_death/high_income_leading_causes_of_death.txt-Krona.html

[I] Siddhartha Mukherjee: *The Emperor of All Maladies*, p.290

5. The fallacy of the infectious disease vaccination model of cancer immune therapy

For a century it was believed that the immune response can destroy anything containing foreign substance, such as pathogens or cancer cells. Following the success of vaccines against xenogeneic infectious diseases, the tacit assumption therefore was that host immunity would also be protective against isogeneic cancer. Although, conventional cancer immunotherapy trials conducted with the best available science resulted in anecdotal responses, theorists contend that neoantigens revealed by next-generation sequencing in cancer cells will be recognized as "foreign" by T cells, in much the same way as T cells sense microbes [24]. In our view, this assumption is fallacious for several reasons out of which we would mention only two.

Large groups of microorganism carry pathogen-associated molecular patterns (PAMPs) that have immunostimulatory activity, which are absolutely essential for the microbe's survival and are therefore invariant structures [25]. The basic machineries underlying innate immune recognition of pathogens are highly conserved among species, from plants and fruit flies to mammals. PAMPs are recognized by pattern recognition receptors (PRRs) of antigen presenting cells (APCs) of the innate immune system. Most spontaneous human tumors, in contrast, have no such distinguishing immune targets. Furthermore, the growth kinetics of tumor cells and infected cells are very different. Even a fast growing tumor with a doubling time of 10 days, will produce less than 10 tumor cells in a month [19], whereas 10^{11} hepatocytes are infected during the same time period (see above). Moreover, established tumors induce tolerance rather than immunity, further weakening the standard tumor immune hypothesis [26].

Unfortunately, virtually all evidence that tumors are capable of shifting the balance of immunity from surveillance to tolerance is circumstantial. Notwithstanding the paucity of data, if one considers the lack of PAMPs on tumor cells and their slow growth, one plausible explanation is that human tumors are unable to activate a sufficient number of APCs, or APCs are less efficient in responding to isogeneic variants to promote IL-2–dependent co-stimulation of T cell before tolerance will have been established [26].

6. Tolerance breakdown is required for eradication of isogeneic tumors

Studies initiated by James Allison led to the breakthrough of treating a variety of malignancies by a prolonged overstimulation with immune checkpoint blockade. This is accomplished with antibodies that target negative regulators of T-cell activity, such as the cytotoxic T lymphocyte-associated antigen 4 (CTLA-4) and the programmed cell death protein 1 pathway (PD-1/PD-L1) [27]. CTLA-4 receptors of T cells are an indispensable braking mechanism on T cell activation to ensure tolerance to self-tissues. Should CTLA-4 not function due to a genetic deficiency or if it is blocked by various manipulations, CD28 functions unopposed would tend to swing the balance in favor of immune stimulation, resulting in the breakdown of tolerance. Anti-CTLA-4 antibody (ipilimumab) improved survival of metastatic melanoma patients by disabling the brakes of T cells. Thus, the price we pay for reversing immunosuppression in cancer by a prolonged immune checkpoint blockade is the generation of uncontrolled T-cell activation. And the direct consequence of this dysregulation is the unleashing of autoimmunity [8].

7. Therapeutic paradigm shift – Proof of principle demonstrated in stage IV cancer

We have proposed a therapeutic paradigm shift. The task is not to put the genie back in the bottle by immune suppressive treatments, but rather harnessing the liberation induced by the anti-CTLA-4 antibody blockade by focusing immune attack: pre-target or reduce the dose of

immune checkpoint blocking drugs [11]. This is consistent with the prediction of Topalian et al. that anti–CTLA-4 and anti–PD-1 checkpoint inhibitor antibody combination require careful dose titrations to define windows of clinical efficacy that does not generate additive or synergistic immune toxicities [28]. Clinical findings from our group support this approach[J]. A patient with stage IV heavily pre-treated triple negative breast cancer (TNBC) with far advanced pulmonary metastases and severe shortness of breath, in whom all conventional therapies had failed was treated with a safe, off label low dose immune checkpoint blockade therapy (ipilimumab 0.3 mg/kg with nivolumab 0.5 mg/kg), complemented with high dose interleukin 2 under taurolidine protection and loco regional- and whole body hyperthermia (without classical chemotherapy). She achieved complete remission of her lung metastases and all cancer related symptoms vanished in the absence of any therapy-associated dangerous autoimmune side effects. A total gene expression analysis of a metastatic axillary lymph node demonstrated that several checkpoint genes were over-expressed, even after one year of the initiation of therapy.

These results are consistent with the 'cancer-immune set point' theory of Chen and Mellman [29], which posits that factors of anticancer immunity are distributed in a continuum and act to determine the so-called cancer immune set point by directly or indirectly controlling the expression of tolerogenic or immunogenic cytokines and cell types. In our view, autoimmune T-cells induced by a low dose immune checkpoint blockade could be a powerful therapeutic tool capable of shifting the balance towards inflammation and immunity. Indeed, our low dose immune checkpoint blockade combination therapy seems to be very close to the ideal cancer immune therapy of Cohen, which results from a regulated activation of a state of autoimmune homuncular war targeted against the tumor [30]

Since our protocol consists only of approved drugs and treatments, our results can be confirmed or refuted in controlled clinical trials.

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Conflict of interest

T.B. is shareholder and CSO of Pret Therapeutics Ltd and Lodoco Ltd.

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