Examining the cooperativity mode of antibody and CD8$^+$ T cell immune responses for vaccinology

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Abstract

A fundamental unsolved issue in vaccine design is how neutralizing antibodies and cytotoxic CD8$^+$ T cells cooperate numerically in controlling virus infections. We hypothesize on a viewpoint for the multiplicative cooperativity between neutralizing antibodies and CD8$^+$ T cells and propose how this might be exploited for improving vaccine-induced protective immunity.

The ongoing SARS-CoV-2 pandemic reminds us about the devastating potential of pathogenic viruses for our well-being and the importance of protective vaccines to keep them under control. Indeed, today, vaccines are among the most efficient and cost-effective weapons to combat infectious diseases. Following immunization, upon reinfection, a pathogen encounters an increased number of pathogen-specific antibodies and antigen-specific T lymphocytes, and the race -- i.e. numbers game [1] -- between pathogen expansion and immune-mediated pathogen elimination is shifted in favor of the host. As a consequence, the vaccinated individual is, in the best-case scenario, either protected from infection or from severe disease [2].

Due to significant progress in (i) virus structure determination by cryo-electron microscopy [3], (ii) rapid and massive sequencing technologies that enable the timely characterization of emerging viruses and the analysis of systems responses upon vaccination (systems vaccinology)[4], (iii) the introduction of mRNA-based vaccines [5] and (iv) the understanding of immune-regulatory mechanisms and network regulations [6,7], a transformation towards rational vaccine design strategies has recently taken place [8,9]. However, in vaccinology, many questions and major challenges remain to be robustly addressed and a more complete understanding of the relationships and cooperativity between humoral and adaptive immunity in response to vaccines is imperative.

With the aim to empower vaccines based on a more complete engagement of different immune mechanisms, in this Forum article we suggest that vaccine protection thresholds may be optimized by exploiting the cooperativity between humoral and adaptive immune response components. We hypothesize that upon vaccine administration, neutralizing antibodies and cytotoxic $CD8^+$ T lymphocytes (CTL) might contribute to protective immunity against viruses in a multiplicative way. Indeed, the role of CTLs in vaccine-induced protection remains to be more fully characterized [6], and their quantitative relationship with antibody responses for vaccine success remain unclear. Based on theoretical grounds, it seems likely that they amplify/synergize their effects, and presumably, this might occur in a non-linear way, although it might also be species- and/or vaccine- dependent. We posit that this question may be relevant for vaccine development as it might help define certain threshold

requirements to achieve protective immunity. Here, we argue that it might be partly answered using a theoretical model that aligns with recent experimental observations. However, future and robust experimental and clinical trials are evidently needed to further examine and validate this model-based prediction of multiplicative cooperativity and its relevance for vaccine design.

Immune protection requirements against virus infections

A simple, well-accepted basic model of virus infection dynamics considers free virus particles (V), uninfected but susceptible target cells (C), and virus-infected cells (Ci) (Figure 1A)[10]. The dynamics of the overall infection process can be expressed in 3 nonlinear differential equations describing the turnover of V, C and Ci with the respective **production rates** α_v, α_c, α_{ci} and **elimination rates** δ_v, δ_c, and δ_{ci} (α = alpha; δ = delta; Figure 1A). Virus propagation i.e. virus infection and expansion within an infected host occurs when the basic **virus reproduction ratio** $R_0 > 1$ while virus is contained if $R_0 < 1$ [10,11]. Neutralizing antibodies inhibit the infection of susceptible target cells C which would increase δ_v, while CTLs kill infected cells C_i which would increase δ_{ci} [10]. As both parameters appear in R_0 as a product, neutralizing antibody and CTL responses reduce R_0 values in a multiplicative way and therefore, both adaptive immune responses synergize rather than simply sum up in virus inhibition [12]. This type of synergistic relationship was initially revealed when both arms of the immune response were first considered in a mathematical model of antiviral immune responses against Influenza A virus infection [12]. Analysis of the **stability condition for an infection-free steady state** [12], which is equivalent to $R_0 < 1$, indicated the multiplicative effect of CTLs and neutralizing antibodies for the elimination of a virus infection. Thus, instead of increasing a single arm of immunity by N-times (i.e. via vaccination), the same protective effect might be achieved by a parallel increase of both arms by $\sqrt{\boldsymbol{N}}$ -times. For example, increasing an antibody titer by 100-times would be equivalent to a 10-times simultaneous rise of antibodies and CTLs in this model. We argue that these considerations might provide a rationale when aiming to overcome the quantitative limitations of single-arm immune response-oriented vaccines.

How do these considerations relate to real-world virus infections? For illustration purposes, by analyzing acute human infections with Influenza A virus (IAV) and Hepatitis B virus (HBV), calibrated mathematical models had been generated that have estimated the different growth and elimination parameters for both infections [12,13]. This has enabled the quantification of the contribution of individual branches of the immune response for virus control (Figure 1B). For IAV with an R_0 value of around 32, the net elimination rates (Figure 1A) of virus (δ_v) and infected cells (δ_{ci}) should be increased such that their product is 32 times larger than the respective products in a naïve host [12]. Likewise, for HBV with an R_0 value of around 4, the product of the corresponding elimination rates should be increased over 4-times. With the described proportions of the elimination terms (see Figure 1B and supplementary table S1), one can now estimate the threshold requirements to reduce R_0 below 1 for either an increase in single arm or combined arms of immunity [13]. A 36-times reduction of R_0 in the case of IVA would require increasing IAV-neutralizing antibody titers by 166-times. The same R_0 reduction could be achieved by an 830-times increase of IAV-specific CTL numbers, or the simultaneous increase of both arms by 25- and 120-times, respectively. The equivalent calculation for a 4-times reduction of R_0 for HBV gives required increases of 52-times for HBV-specific antibodies, 240-times for HBV-specific CTLs and a combined 18- and 80-times increase for both, respectively (summarized in figure 1C).

The theoretically-derived estimates of the immune threshold conditions for virus control ($R_0 < 1$) can be corroborated by empirical virus infection data for which calibrated mathematical models do not exist. In a recent study, data were provided for vaccinated rhesus macaques (15 animals per immunization group) with either a neutralizing antibody-inducing or a neutralizing antibody- plus CTL-inducing vaccine against simian–human immunodeficiency virus (SHIV) infection [14]. The combination of neutralizing antibody and CTL induction reduced the threshold requirements for neutralizing antibodies to confer protection. Animals with a mean neutralization infectious dose 50 (ID50) titer value of 800 remained uninfected upon SHIV challenge while animals with a mean neutralization ID50 titer value of 50 became infected (see [14], extended data Fig. 8). A mere 2.5-times increase from 0.1% to 0.25% of virus-specific CTLs protected the animals with the low antibody titer (see [14], extended data Fig. 9a). Thus, a 16-times decrease of the neutralizing antibody titer might be compensated by just a 2.5-times increase in the number of CTL to confer protection against infection. Taken

together, the induction of neutralizing antibody responses and CTL could reduce the required protective titer of antibodies by more than an order of magnitude (Figure 1D).

The current approaches of vaccine design concentrate on the definition of immunogenic epitopes, adjuvants, delivery routes and vaccine formulations. While all these are fundamental elements of vaccines and their success, the multiplicative cooperativity of the humoral and cellular arms of the adaptive immune response highlights another consideration for vaccine design and predicting possible response outcomes, namely, that both immune arms should be induced to exploit their synergy including their time-deferred mode of cooperation [15]. Indeed, we posit that this concept might provide a basis for understanding why attenuated vaccines can be so efficient whereas subunit vaccines that focus on inducing neutralizing antibody responses might lack sufficient epitopes to achieve robust cellular immunity and thus, might result in suboptimal protection. However, this concept remains conjectural and the complexity of vaccine responses cannot be understated. Nevertheless, we propose that exploiting the concept of multiplicative cooperativity might be useful when aiming to reduce the protective immune threshold requirements of a successful vaccine. A lower threshold might then perhaps also help in controlling a wider spectrum of virus variants that appear during outbreaks and that might need more stringent immune responses. We anticipate that further data-driven and hypotheses-oriented modeling studies might assist in these endeavors and are clearly warranted.

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Figures

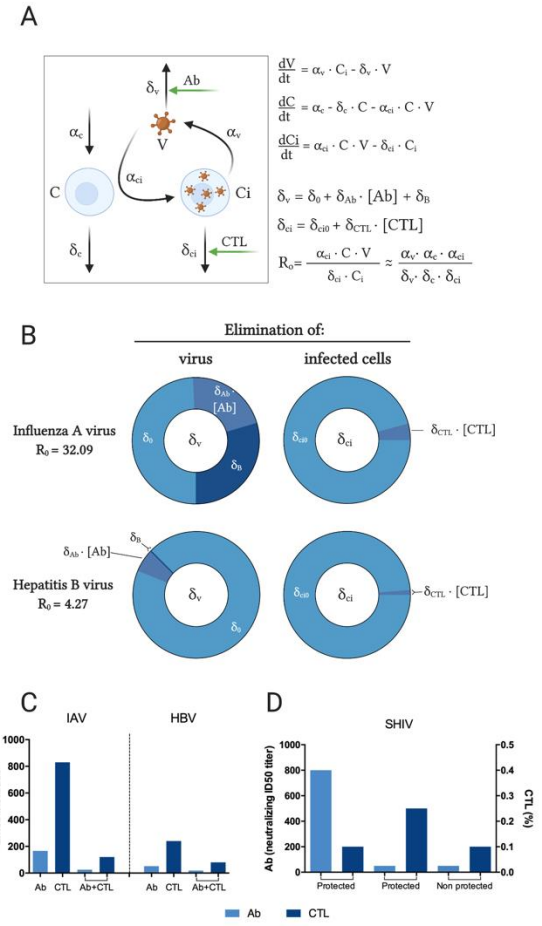

Figure 1. Illustration of a mathematical model for fundamental immune interactions providing protection against virus infections. (**A**) Basic model of virus infection dynamics at the initial phase of infection. It describes the rate of changes ($\frac{d}{dt}$) of the population densities of free virus particles (V), susceptible target cells (C) and virus-infected cells (Ci). The balance of the growth and elimination processes determining their dynamics are described in the right-hand-sides (r.h.s.) of the equations. The intensities of these processes are characterized by the respective **production rates** α_v, α_c, and α_{ci} and **elimination rates** δ_v, δ_c and δ_{ci}. The net per capita elimination rate of the virus population is a sum of the natural degradation rate δ_0, antibody-mediated elimination δ_{Ab}[Ab], and the fraction of virus bound to target cells δ_B which then is unavailable for infecting other target cells. The net per capita elimination rate of the infected cell population is a sum of the natural death rate and CTL-mediated

elimination, denoted by δ_{C0} and δ_{CTL}[CTL], respectively. The levels of vaccine-induced antibodies and CTL are denoted by [Ab] and [CTL], respectively. Green arrows indicate their protective contribution to the elimination of an infection. The multifactorial parameter R_0 characterizes the relative balance of infection spreading versus elimination. It can be defined as the ratio between cell infection rate and infected cell elimination rate (r.h.s. of the third equation in fig. 1A), estimated soon after an individual got infected. Because of this time element in the definition of R_0, the third term on the r.h.s. of the second equation is neglected so that $C \approx \frac{\alpha_C}{\delta_C}$. Using the quasi-steady-state approximation for the viral load $V \approx \frac{\alpha_V}{\delta_V} Ci$, we arrive at the displayed expression for R_0. (**B,C**) Characteristics of IAV and HBV infections of non-vaccinated human individuals according to calibrated mathematical models of both infections [12,13]. **(B)** R_0 values as well as relative values of virus and infected cell elimination parameters. Numerical parameter values are in supplementary material Table S1. The units of all parameters δ are 1/day. (**C**) Hypothetical thresholds of antibody, CTL and antibody plus CTL required for protection against IAV and HBV infection. Given are the times increase of required amounts of virus-specific antibodies (Ab) and/or virus-specific CTL with respect to a non-vaccinated naive human. (**D**) Hypothetical SHIV-specific neutralizing antibody concentrations (in neutralization infectious dose 50 (ID50) titer values) and CTL numbers (in percentage) that may protect or not protect against a SHIV challenge of rhesus macaques based on [14]. This figure was created with BioRender.com.

Glossary

Production rate α -number of produced (target cells, infected cells, virions) over a given time period.

Elimination rate δ - number of eliminated (target cells, infected cells, virions) over a given time period.

Virus reproduction ratio R_0 – the relative balance of infection spreading versus elimination within an infected host. If $R_0 > 1$, a virus infection spreads, while it is eliminated if $R_0 < 1$.

Stability condition for an infection-free steady state – a condition under which a virus infection is cleared. This condition is mathematically expressed as an inequality relationship on the rates of production and elimination which determines that the virus-free state is stable.

Table S1.

Characteristics of IAV and HBV infections of non-vaccinated human individuals according to calibrated mathematical models of the infections [12,13]. Given are the R_0 values for both infections as well as relative values of virus and infected cell elimination parameters. The units of all parameters α and δ are 1/day. The intensities of the respective processes are characterized by the respective **production rates** α_v, α_c, and α_{ci} and **elimination rates** δ_v, δ_c, and δ_{ci}. The net per capita elimination rate of the virus population is a sum of the natural degradation rate δ_0, antibody-mediated elimination δ_{Ab}[Ab], and the fraction of virus bound to target cells δ_B which then is unavailable for infecting other target cells. The net per capita elimination rate of the infected cell population is a sum of the natural death rate and CTL-mediated elimination, denoted by δ_{C0} and δ_{CTL}[CTL], respectively. The levels of vaccine-induced antibodies and CTL are denoted by [Ab] and [CTL], respectively.

Parameter	Values for IAV	Values for HBV
$R_0 = \frac{\text{Growth}}{\text{Elimination}}$	32.09	4.27
$\alpha_v \cdot \alpha_c \cdot \alpha_{ci}$ (Growth)	174.4	0.0954
$\delta_v \cdot \delta_c \cdot \delta_{ci}$ (Elimination)	5.4043	0.0224
$\delta_{ci} = \delta_{ci0} + \delta_{CTL} \cdot [CTL]$ (Elimination of infected cells)	1.566 = 1.5 + 0.066	0.0527=0.052+0.00066
$\delta_v = \delta_0 + \delta_{Ab} \cdot [Ab] + \delta_B$ (Elimination of free virus)	3.45=1.7 + 0.731+1.02	0.4249= 0.4 + 0.0249+0.0000125