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A quantitative model of autoimmune disease and T-cell vaccination: does more mean less?

Lee A. Segel, Eva Jäger, Dana Elias and Irun R. Cohen

According to a simple mathematical model, the activated effector T cells that cause an autoimmune disorder can also cure the disease if administered in large doses. This prediction has been tested in the nonobese diabetic (NOD) mouse model and demonstrates that administration of intermediate doses of a diabetogenic T-cell clone caused early hyperglycemia, whereas a higher dose cured the disease. As discussed here by Lee Segel and colleagues, the proposed application of T-cell vaccination to treat clinical disease obliges immunologists to consider the quantitative complexities of regulation.

The ability of clones of T cells to cause experimental autoimmune encephalomyelitis (EAE) is influenced by their quantity as well as by their quality. The latter is determined by the specificity of their receptors for myelin basic protein (MBP) and their state of activation, including the nature and amount of cytokines and other biological effector molecules they express. A virulent anti-MBP T-cell clone will produce EAE if, and only if, a suitable number of cells is administered. For example, inoculation of rats with 10^7 activated anti-MBP T cells can cause lethal EAE; 10^6 cells can cause severe, but not lethal, disease; and a dose of 10^5 cells may cause only mild disease[1,2]. Administration of 10^4 or fewer anti-MBP T cells may produce no disease at all. This association of the severity of EAE with the number of virulent T cells is not entirely unexpected. What is

surprising is the observation that administration of 10^4, 10^3, or even 10^2, activated anti-MBP clone cells can induce 'vaccination' – that is, a state of resistance to subsequent challenge with lethal doses of the same clone[1].

The induction of resistance to autoimmune disease by administration of subpathogenic doses of otherwise virulent autoimmune T cells has been called low-dose T-cell vaccination[1–3]. In early studies, T-cell vaccination was obtained using activated T cells that had been attenuated either by irradiation[4], by hydrostatic pressure or by chemical crosslinkers[5]. These methods of attenuation caused qualitative changes in the T cells. Thus, it might be anticipated that administration of even large numbers of such modified T cells might induce resistance to disease rather than disease itself. But why should low-dose vaccination with unmodified autoimmune T cells, which should appear as self to the immune system, induce resistance to lethal doses of the same cells?

Vaccination induces regulation

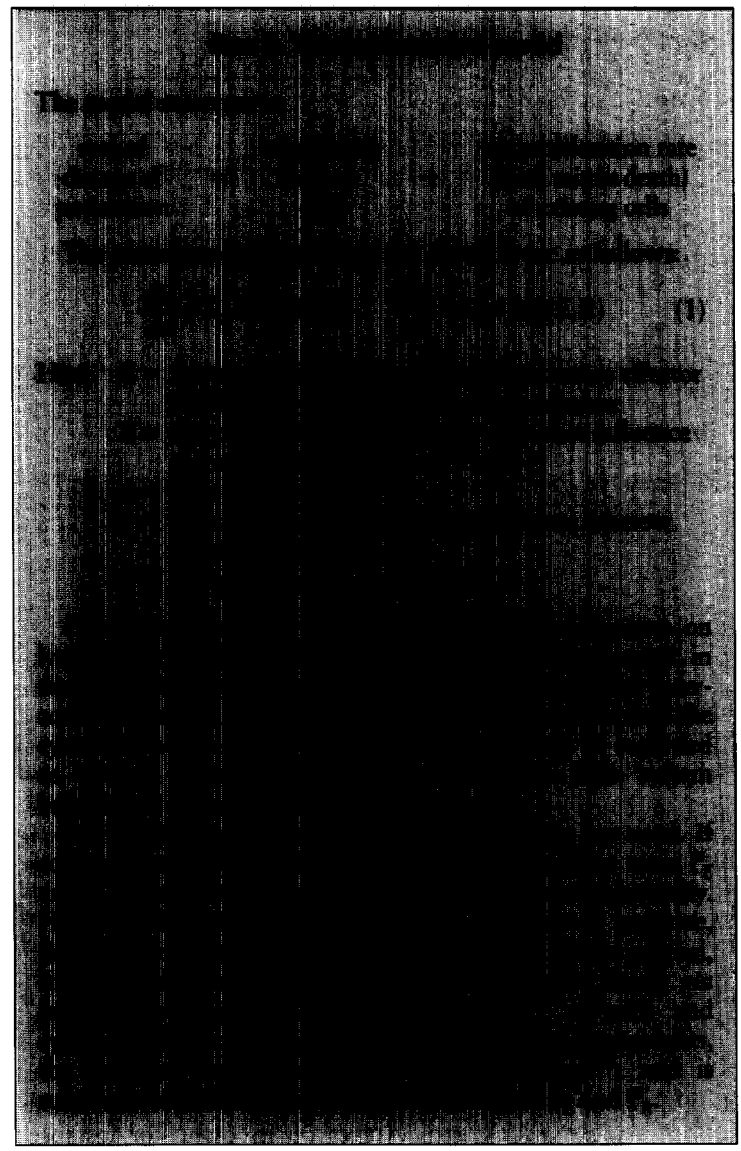
The answer to the question posed above appears to be related to the observation that low-dose T-cell vaccination activates anti-idiotypic regulatory T cells: it was shown that a subpathogenic dose of anti-MBP T cells activated anti-(anti-MBP) T cells of both the $CD4^+$ and $CD8^+$ phenotypes[3]. The presence of these anti-idiotypic T cells was associated with resistance to a lethal dose of anti-MBP T cells[2,3]. Furthermore, a trial of T-cell vaccination in patients with multiple sclerosis (MS) demonstrated that downregulation of anti-MBP T cells was associated with upregulation of anti-idiotypic T cells[6]. Thus, with the advent of human trials, models that clarify the dose–response relationships of T-cell vaccination and autoimmune disease would be helpful in planning protocols.

The appearance of regulatory T cells within five days of vaccination with as few as 10^2 anti-MBP T cells led to the hypothesis that networks of regulatory T cells, specific for the anti-MBP T cells, existed naturally in healthy individuals even before the induction of EAE. The presence of 'sets' of autoimmune lymphocytes and regulatory lymphocytes was proposed to constitute the internal image held by the immune system of some of the immunologically dominant self-antigens, including MBP, heat shock protein 60 (Hsp60) and others. This internal image of natural autoimmunity and its regulation was termed the 'immunological homunculus'[7–11].

An automaton model of T-cell regulation was developed based on the operation of the homunculus[12]. However, a paradox remains: given the quantitative nature of T-cell vaccination, how can a decrease in the dose of an injected clone of T cells later result in a completely different state of the animal? Indeed, given that the larger dose induces disease, how can one explain – without invoking a change in quality – how the smaller dose leaves the animal not only free from disease, but also vaccinated against future attacks of disease?

A model to clarify dose-dependent regulation

Our goal here is to demonstrate that a mathematical model can resolve the quantitative paradox described above. In so doing, we construct a minimal model of the autoimmune homunculus. It will become clear that specific, and sometimes unexpected, experimentally testable predictions result.



The model describes the interaction of two cell types. The first of these, labeled E, represents the population of dominant autoimmune effector T cells that cause an autoimmune disease. Since the homunculus paradigm[11] asserts that 'dominance is welded to regulation', a minimal model must contain a regulatory population, R. The cells R (a simplified grouping together of several actual populations) influence the effector cells E and, in turn, are influenced by them. This mutual influence may work by idiotype–anti-idiotype interactions[13], although it could also be mediated *via* cytokines triggered by antigens or other ligands[14]. B cells may even be involved[15]. At this stage, the model attends only to the broad outlines of relations between its elements and to the organization of their interactions; it has nothing to say about the chemistry that ties the structure together. The mathematical model is described in Box 1.

Models, like balls rolling on a landscape, tend to different steady states

Here, we shall consider populations E and R, whose contact with the outside world is limited to a single

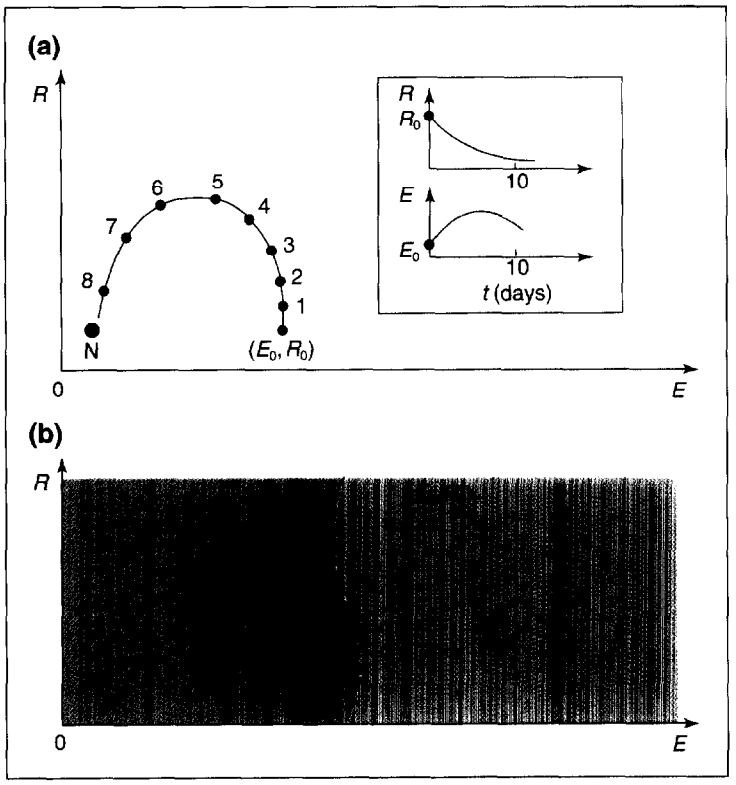


Fig. 1. *(a) Possible trajectory of effector (E) and regulatory (R) cell populations. Numbers indicate days when populations were measured, starting with initial conditions (E_0, R_0) at day zero and approaching ever closer to the normal steady state N. Inset: corresponding conventional graphs of E(t) and R(t) where t = time. (b) For a hypothetical model, the set of initial conditions that result in diseased steady state D, rather than N, are shaded blue and pink, respectively. Thus, blue and pink regions form the domains of attraction of D and N, respectively. One typical trajectory (starting from E_0, R_0) is also shown, with an arrow indicating the direction of motion as time increases. The dashed line separating sets D and N represents the separatrix.*

and virtually instantaneous initial event, after which the (*E*, *R*) system evolves on its own. For instance, such an event might be represented by the administration of a dose of *E* cells, or the endogenous development of autoimmune effector cells following an infection or an accident. Initial events lead to 'initial conditions' of the populations, wherein these populations are prescribed as E_0 and R_0 at some initial time (taken to be t = 0).

The mathematics of systems such as that described in Box 1 allow us to make the following statements: (1) given any initial state (E_0, R_0), the future course or 'trajectory' of *E*(t) and *R*(t) is uniquely determined; (2) after transients die out, *E*(t) and *R*(t) approach an 'attractor' – which can be either a steady state or a continuing periodic oscillation (Fig. 1a) (the latter alternative will not be considered here); and (3) since more than one steady state may be possible, the steady state that is approached depends on the initial conditions.

Thus, if one considers a billiard ball rolling on a smooth landscape with several pits, how the ball rolls is determined by the form of the landscape, and this represents the proliferation functions. The geographic position (state) of the ball at any time [i.e. its (*E*, *R*) coordinates] corresponds to the momentary values of the *E* and *R* cell populations. Each pit corresponds to a possible (stable) steady state. After being jostled, the (*E*, *R*) system, like a billiard ball, will move into a position (state) that depends both on its initial position (state) and how it was jostled. Thus, any new stable relationship between autoimmune effector cells and regulatory cells will depend on their state at the moment that the initial relationship was upset. The new state will also depend on how the initial state was upset.

Suppose that, for a certain set of circumstances, exactly two steady states can be attained (stable states), one of which is a normal state, *N*, and the other a diseased state, *D*. Thus, *N* would represent a state of health (i.e. no overt autoimmune disease) and *D* would represent a clinical disease. As shown in Fig. 1b, the set of all possible initial conditions is divided into two 'domains of attraction' – those conditions resulting ultimately in state *N* and those resulting in state *D*. The boundary that separates these sets is called a 'separatrix'. Thus, the model structure indeed embodies essences of the homunculus paradigm, tenets of which are that 'by deploying built-in information [the proliferation functions P_E and P_R] the system knows what it is looking for [one of the two steady states *N* and *D*]'[10]. Which of the two restricted responses (*N* or *D*) is selected depends upon experience (comprising the past history embodied in the proliferation functions, and the initial conditions).

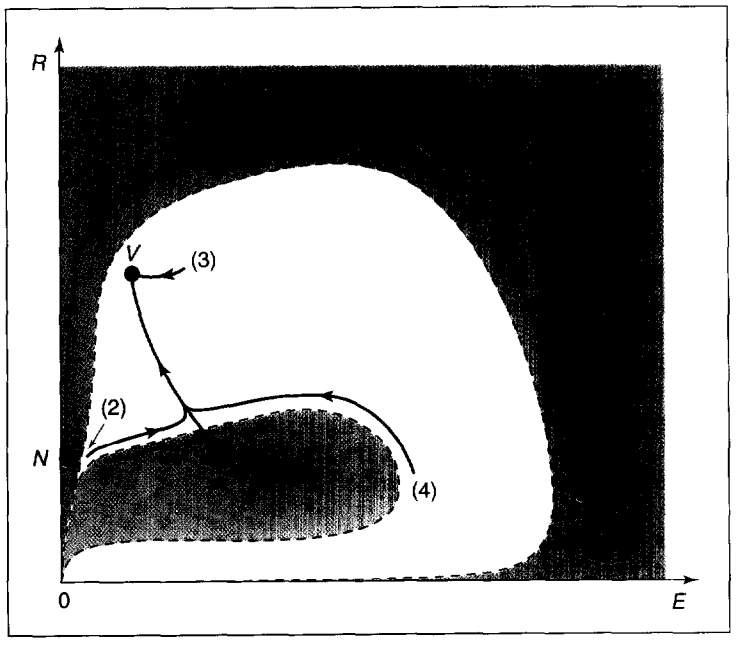
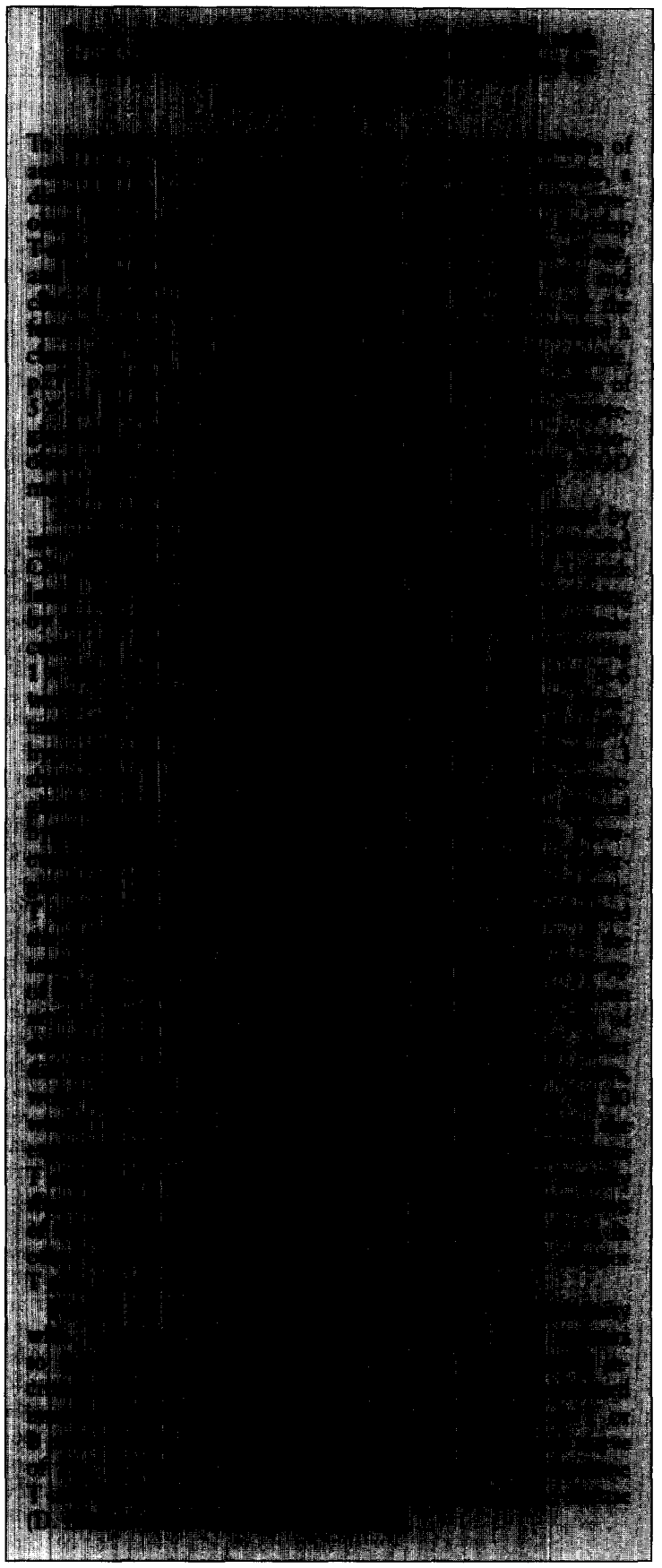


Fig. 2. *Diagram of a particular case of the model of Eqn 1 for T-cell vaccination (see Box 1). There are three steady states: normal (N), vaccinated (V) and diseased (D), with domains of attraction (separated by dashed separatrices) that are respectively pink, white and blue. Trajectories for initial conditions (1)–(5) are indicated; the proliferation rates were chosen to illustrate major known findings concerning T-cell vaccination with live cells. Trajectories represent: (1) a relatively large dose of effector cells leads to disease; (2) a suitably smaller dose of effector cells leads to vaccination; (3) once the animal is in a vaccinated state, the same dose that would previously have led to disease now no longer does, and the animal returns to a healthy vaccinated state; for (4) and (5), see text. For the differential equations that were integrated to obtain Fig. 2, and for considerably more theoretical detail, see Ref. 18.*



'Normal', 'diseased' and 'vaccinated' are modeled as steady states

To apply the ideas described above to the situation of T-cell vaccination, we consider the possibility of three stable steady states – *N*, *D* and vaccinated (*V*), with their respective domains of attraction bordered by separatrices. *V* means that the individual is resistant to a dose of autoimmune effector cells that would have produced disease in that individual before vaccination, in the normal state. It is possible to construct a family of differential equations whose behavior is immunologically reasonable; the equations describe what happens when we carry out low-dose T-cell vaccination. One example is illustrated in Fig. 2. Many observed properties of *N*, *D* and *V* states are illustrated in the figure (see caption). Note that the population of autoimmune effector cells is considerably higher than normal in the *V* state. However, disease is avoided, since these cells are prevented from further expansion by a high population of regulatory cells. The resistance to autoimmune disease that develops following the administration of the self-antigen[16,17], or following T-cell vaccination with dead or treated T cells, can be modeled by adding a suitable differential equation to Eqn 1 (Box 1)[18].

Different T-cell doses can induce transitions between steady states

At this point, the reader may wonder why they should bother to read about equations that merely reproduce what we know from experimental results that are already described in the literature. However, the chief value of a model is to formulate predictions that can prompt experimentalists to do experiments that they might not have otherwise done. For example, the model suggests that high doses of attenuated or dead T cells can yield disease. Thus, the model alerts us to the possibility that T-cell vaccination, even using dead vaccines or T-cell receptor (TCR) peptides, may be hazardous. Indeed, de Alboran *et al.* have already demonstrated this point inadvertently: a dose of 2.5×10^5 irradiated T cells will vaccinate MRL-*lpr* mice against the development of spontaneous systemic lupus erythematosus (SLE), whereas 5×10^6 of the same attenuated T cells does not protect and may even enhance disease[19]. Thus 'overvaccination' can indeed occur, as predicted by the model.

The model shown in Fig. 2 makes another counter-intuitive prediction: that administration of a large dose of live effector T cells *E* to an already diseased individual can trigger the system to move toward the *V* state and, hence, result in a cure [see the trajectory beginning at (4); note that a very large dose of *E* cells to a normal individual should also vaccinate]. Moreover, for this version of the model, an even larger dose will induce what can be termed 'overcure', by triggering the system to move toward the *N* state [trajectory (5)]. In other words, a large number of autoimmune effector cells could have the paradoxical effect of actually turning off the disease. For a demonstration of this implausible prediction in an experimental situation, see Box 2. Perhaps such 'high-dose tolerance' is not so unusual when one considers the fact that some patients can enjoy long remissions, even cure, after an acute attack of a disease such as MS. Indeed, self-curing autoimmune disease may be much more common than we suspect, since individuals suffering a single bout of an autoimmune disease may escape medical attention.

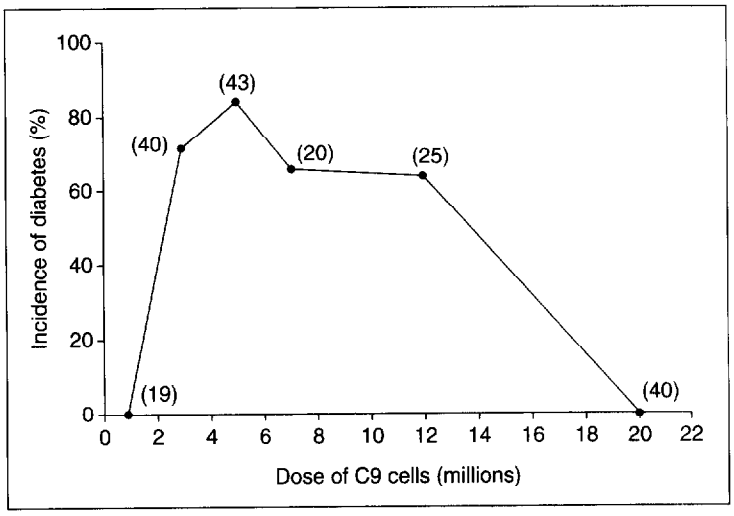


Fig. 3. *Dose–response relationship of hyperglycemia produced by a T-cell clone. The curve summarizes two experiments that produced essentially identical results (see Box 2). The diabetogenic CD4$^+$ T-cell clone C9 was activated as described[17] and female nonobese diabetic (NOD) mice of age 5–6 weeks were inoculated intraperitoneally with various numbers of cells. One week later, the mice were scored for significant hyperglycemia (greater than 3 SD above the normal). The appearance of spontaneous diabetes at 30 weeks of age was also scored, but is not shown here. The numbers of mice receiving each dose are shown in parentheses.*

Concluding remarks

The predictions made by our model are governed by the positions of the separatrices, which in turn depend on details of the *E–R* interactions. Since these are not known, and in any case would vary in different genotypes, it is instructive to consider possibilities beyond the scope of this article[18]. From a general perspective, the effects of T-cell vaccination can be described as a strengthening of connections in the protective network[8–12]. With its stress on the domains of attraction, the present model sharpens this image by indicating that suitable perturbations of a system can cause it to cross thresholds in the immune landscape and enter new steady states. Our model suggests several unusual experiments, some of which, as we have seen, have already been successfully carried out: for instance, de Alboran *et al.* have shown that vaccination may be compromised if too many cells are used and, as we have shown in Box 2, vaccination with increased numbers of autoimmune effector T cells may shut off an autoimmune disease.

Whether verbal or mathematical, a model cannot be proven to be correct even if its predictions turn out to be accurate; many other models might make the same predictions. By a similar logic, negative results cannot invalidate a model; there are untold reasons to account for failed experiments. But the degree of belief in the truth or falsehood of a model ultimately depends on the results of experiments. Thus, especially for conceptual models such as ours, the value of a model may lie not so much in its falsifiability[20] as in the experiments it makes thinkable.

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