Theoretical Article

Some Scaling Principles for the Immune System

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Summary Using recent progress in biological scaling, we explore the way in which the immune system of an animal scales with its mass (M). It is shown that the number of cells in a single clone of B cells should scale as M and that the B-cell repertoire scales as $ln (cM)$, where c is a constant. The time that a B cell needs to circulate once through the organism is shown to scale as $M^{1/4} ln (cM)$. It is suggested that the scaling of other cell populations in the immune system could be derived from these scaling relations for B cells.

Key words: protectons, scaling laws, theoretical immunology.

Introduction

We explore the notion that the immune system of mammals might show some features that obey simple scaling laws. Such a conjecture seems quite plausible in view of the preponderance of scaling laws throughout most subdisciplines of biology, where they are usually called allometric relations.

To be more specific, one could compare the immune systems of the following organisms: tadpole (body mass of the order of $10^{-1} g$), hummingbird ($1 g$), mouse ($10 g$), man ($10^5 g$), elephant ($10^6 g$) and whale ($10^8 g$). How is the increase in body weight reflected in the design of the immune system? Two points of view suggest themselves here, which we shall call modular and global.

Langman and Cohn[1] suggested that the immune system has a modular structure and is built of basic units called 'protectons' that guarantee an adequate immune response. A big animal simply has more protectons in its immune system than a small animal. They estimated, based on the concentration of antibody needed to protect an animal, that a protecton contains about 10^7 B cells in a volume of about 1 mL. In terms of scaling, the protecton idea suggests that the size of the immune system scales as the mass of the organism.

The second point of view conjectures a global structure for the immune system. The whole system is considered as a single 'reaction vessel', which is stirred continuously by the action of the heart. In this view the movement of lymphocytes throughout the body is emphasized. When an antigen enters the body through the skin it is usually transported to the draining lymph node and a local reaction ensues in which the lymph node swells. Interestingly, the majority of lymphocytes specific for that antigen in the circulation can accumulate in the draining lymph node. Thus, in this regard the global trafficking of lymphocytes is important.

Here we will not adopt either viewpoint, since the immune system seems to have some features that appear to be modular and others that appear to be global. Rather, we will focus on questions related to how the immune system scales with the mass of an animal. Scaling should help reveal those aspects that are modular and those that are global in character.

A larger animal has more B and T lymphocytes. This implies either more lymphocyte clones, or more cells per clone, or both. This suggests the question: what is the optimal way for the system to balance these two modes of resource allocation – T and B cell diversity versus clone size? In the following we pursue this question, but we first ask what determines the average lifespan of an organism, of a mammal for example?

The lifespan of a mammal is related to its mass

Empirical evidence suggests that the average lifespan (T_0) of a mammal scales with its body mass (M) according to the scaling law $T_0 \sim M^{1/4}$, cf.[2] A scaling law between a biological variable Y and body mass M is written in the form $Y \sim M^b$, and b is called the scaling exponent. This is shorthand for an approximate, quantitative relation

$$\frac{Y}{Y_0} \cong A\left(\frac{M}{M_0}\right)^b, \tag{1}$$

where Y_0 is a standard unit with the same dimension as Y, M_0 is a standard unit of mass, and A is a dimensionless constant.

Given the empirical observation that lifespan scales $\sim M^{1/4}$, it would seem that the immune system must have evolved to protect an organism for times that scale $\sim M^{1/4}$. The important point is that the immune system of a larger animal must help keep that animal alive for longer periods than the immune system of a smaller animal, and hence must be more reliable. Thus, while the principles of immunology, such as clonal selection, appear to be universal, the actual details of implementation differ between species. These scaling relations suggest that the detailed implementation of immune system principles in larger animals lead to a more reliable system. Whether this is just a question of larger repertoires or other feature is explored below.

Scaling of B and T cell clone size

In order to derive the typical size of a lymphocyte clone as a function of M, we follow the model of West, Brown, and Enquist (cf. [2] and [3], especially pp. 87–112), in which the circulatory system is represented by a branching tree.

In the WBE model the organism is divided into a certain number of small units, each of which is supplied by a single capillary. These units, called *service volumes* or *service units*, are regions that a single capillary can supply with oxygen and remove waste products from. It is shown in [2] and [3] that the number of service units scales $\sim M^{3/4}$. This implies that the volume of a service unit scales as $\sim M^{1/4}$. For the order-of-magnitude estimates that follow, one can assume that a service unit is spherical; its radius (R) will scale $\sim M^{1/12}$.

We assume that the service volumes for the blood circulation are also the service volumes for immune surveillance. That is, the capillaries allow lymphocytes to exit the circulatory system and explore regions of tissue for foreign molecules and cells, collectively called antigens. This implies that if each clone of B cells or T cells contains at least $\sim M^{3/4}$ cells it can be represented in each of the $\sim M^{3/4}$ service volumes.

Now consider what goes on inside a single service unit. One of the essential ideas in the WBE model is that the terminal capillary, which ends in this volume, is universal in its properties such as its diameter. This implies that the amount of blood delivered to the service unit, per unit of time, is independent of M. From the point of view of immune surveillance, most antigens will enter the service unit in this blood. In other words, the number of antigens that enter into the service unit per unit of time is independent of M.

The time to find an antigen in a service unit

Consider a single antigen in the service unit in question – and one specific lymphocyte – of some clone that is specific for the antigen which is also located in the same service unit. This lymphocyte will crawl between the cells and biopolymers of the service unit in a more-or-less random fashion, checking them for antigen. T cells will look for MHC-foreign peptide complexes expressed on the surface of a cell, while B cells will look for antigen, say bacteria, in the intracellular matrix. How long does it take until it makes contact with the antigen for the first time?

If one describes the random walk of the lymphocyte as spatial diffusion with a diffusion coefficient D, then the following dimensional argument suggests itself. Let the antigen be at rest at the centre of the spherical service unit. At time $t = 0$, the lymphocyte at the outer edge, at a radial distance $r = R$, begins searching. Contact between the lymphocyte and the antigen occurs if $r = l$, where l equals the sum of the radii of the lymphocyte and of the antigen (both of which are modelled as small spheres). The average time until first contact (T) can only be a function of D, R and l. Putting

$$T = D^{\alpha} R^{\beta} l^{\gamma} \qquad (2)$$

one finds $\alpha = -1$ and $\beta + \gamma = 2$. Hence the average time until the lymphocyte meets the antigen for the first time must have the form

$$T = \frac{R^2}{D} f\left(\frac{R}{l}\right) \qquad (3)$$

where f is a function of the dimensionless variable R/l, which will be $\gg 1$ in our case. The explicit form of this function was calculated in Appendix I of [4], using earlier results from [5]. The asymptotic form is found to be quite simple

$$f(R/l) \cong \frac{1}{3}(R/l), \ (R/l \gg 1). \qquad (4)$$

We conclude that the average time until first contact between the lymphocyte and antigen is given by

$$T = \frac{R^3}{3Dl}. \qquad (5)$$

The rate of lymphocyte searching

Returning now to the biology of the immune system, one needs to ask whether the diffusion coefficient D depends on the activity of the lymphocyte only, and hence is independent of D, or if it in some way depends on the metabolic activity of the animal and hence on M. We first consider the case in which D is independent of M; then as $R \sim M^{1/12}$, (Equation 5) shows that $T \sim M^{1/4}$. In other words, if there were only one lymphocyte per clone present in the service unit under consideration, the antigen could go undetected by that lymphocyte for a period of time ($\sim M^{1/4}$) that increases with animal size. Because the search time for each clone should scale in the same manner, this result applies to all clones. In order to keep the time until detection a fixed value (smaller than the time during which the antigen could proliferate significantly) the organism has to put $\sim M^{1/4}$ copies of the lymphocyte under consideration into this service unit; this would reduce the mean time until first detection by a factor of $\sim M^{1/4}$ to a value which is independent of M. We conclude that if D is independent of M, the lymphocyte clone size should scale $\sim M^{3/4} M^{1/4} = M$, where the first factor comes from the number of service units and the second one from the number of lymphocytes needed per unit.

Now, we examine the second possibility: the rate at which a lymphocyte randomly searches, characterized by the value of D, depends on the metabolic rate of the organism, and hence its size. The total metabolic rate of an animal scales as $\sim M^{3/4}$, an allometric relation experimentally discovered by Kleiber in 1932 and theoretically explained by the WBE model.[2,3] If the metabolic rate of the lymphocyte population scales as the metabolic rate of the whole animal, then this also scales as $\sim M^{3/4}$. As the size of a lymphocyte is independent of the mass of the animal, this would imply that the metabolic rate of a single lymphocyte scales as $\sim M^{-1/4}$.[6] From this, the scaling property of D follows immediately if one visualizes the random motion of a cell as a succession of jumps, with each jump covering a fixed distance l, and proceeding in a random direction. The time between jumps is denoted by τ. During these short time intervals τ, the cell has to metabolize an amount of energy, E, required to take the next jump. One expects the time of jumping τ multiplied by the metabolic rate of a cell, $\sim M^{-1/4}$, to be proportional to E, that is, $E \sim \tau M^{-1/4}$. As E should depend on the difficulty of moving through the

Table 1 Number of lymphocytes in a single clone

	Antigen reproduces autonomously	Antigen reproduction dependent on organism
Autonomous lymphocyte movement	$\sim M^{3/4}\, M^{1/4} = M$	$\sim M^{3/4}\, 1 = M^{3/4}$
Organism-dependent lymphocyte movement	$\sim M^{3/4}\, M^{1/2} = M^{5/4}$	$\sim M^{3/4}\, M^{1/4} = M$

media, it will (probably) be independent of M. If E is independent of M, then τ will be proportional to $EM^{1/4}$, that is, to $M^{1/4}$. Because the diffusion coefficient is proportional to $1/\tau$, this implies that

$$D \sim M^{-1/4}, \qquad (6)$$

This same relation could also be derived by simply assuming that D is proportional to the metabolic activity of a lymphocyte, $\sim M^{-1/4}$.

Using Equation 5 would give the average time until first contact between an antigen and a lymphocyte a scaling

$$T \sim R^3/D \sim M^{3/12}\, M^{1/4} = M^{1/2}. \qquad (7)$$

This would imply that $\sim M^{1/2}$ copies of this lymphocyte should be in the service unit in order to reduce the search time to a value independent of M. The total size of the lymphocyte clone should now scale as $\sim M^{3/4}\, M^{1/2} = M^{5/4}$ where, again, the first factor comes from the number of service units and the second from the immune detection process that goes on in each service unit.

A third possibility for the lymphocyte search is to assume D to be independent of M, but to assume that the antigen being searched for is a pathogen that depends on host resources for growth, and hence proliferates at a rate proportional to the mass-specific metabolic rate of the organism, $M^{3/4}/M$ or $M^{-1/4}$. As the amount of metabolic energy, which is produced per unit of time and per unit of body mass, scales $\sim M^{-1/4}$, the antigen will need a time $\sim M^{1/4}$ to proliferate. Thus, the immune system will need to find the antigen in a time $\sim M^{1/4}$. As the search time of a single lymphocyte was shown to scale $\sim M^{1/4}$, we conclude that the number of lymphocytes of the particular clone, per service unit, is independent of M.

Finally, we consider the fourth possibility in which both the lymphocyte and the antigen follow the body's metabolism. In this case the average time until first contact between them scales as in Equation 7: $T \sim M^{1/2}$. As the proliferation time of the antigen scales $\sim M^{1/4}$, one needs $M^{1/4}$ copies of the lymphocyte in the service unit in order to keep the antigens in check.

The size of a single lymphocyte clone is displayed in Table 1 for these four possible scenarios. We know of no experimental evidence that would allow us to choose among these possibilities, but it seems most plausible to us to assume that the behaviour of both the lymphocyte and the antigen depend on the availability of nutrients such as glucose, and hence both should be 'hooked up' to the organism's basic metabolism. Thus, we suggest that

$$\text{clone size} \sim M \qquad (8)$$

is the relevant scaling law, and we shall use this law in the remainder of this paper.

Scaling of the lymphocyte repertoire

Next, we ask how many different clones of lymphocytes should be present in an animal of mass M. This number can be estimated in the following way. According to Kleiber's law, the total metabolic rate of the animal scales as $\sim M^{3/4}$. Moreover, the average lifespan scales $\sim M^{1/4}$. Combination of these two relations leads to the result that the total metabolic activity of the animal, during its whole life, is $\sim M$.

This total metabolic activity is the result of the intake of food, liquids and air, and their subsequent treatment by biochemical pathways that are common to all mammals. It seems quite plausible to assume that the total number of infections during the lifespan of the organism is simply proportional to this intake and hence to the lifetime total metabolic activity, that is, it is a number that scales $\sim M$. Thus, we shall denote the total number of infections per lifetime by cM, where c is a constant.

This means that the immune repertoire must be diverse enough to eliminate a total number of infectious agents that equals cM with a probability that is very near to unity. In order to estimate this survival probability, we use the concept of shape space, introduced by Perelson and Oster.[7] For simplicity, we will state the argument for B cells but a similar argument holds for T cells. Each clone of B cells consists of identical cells, each of which carries in its outer membrane a number of immunoglobulin receptors that all have binding sites of a certain shape. This shape (and the other parameters one needs to specify in order to characterize the binding site in an immunological way) is represented by a single point in shape space. If there are N different clones of B cells they correspond to N points in shape space. An antigen that infects the body has regions that the immune system recognizes, called epitopes. Each epitope can also be represented by a point in shape space. Although the B cell that recognizes an epitope should have complementary shape, it simplifies the argument if we think of the B cell that perfectly recognizes an epitope to lie at the same point in shape space. Each antigen may have multiple epitopes, for example, the hemagglutinin molecule on the surface of influenza A has four non-overlapping antigenic regions.[8] However, for the scaling arguments made here, it suffices to assume each antigen has a single epitope. We also assume that there is a (small) volume v_0 around the antigen's representative point, such that the antigen can be eliminated by the immune system if at least one B-cell clone has its representative point inside v_0, that is, recognition need not be perfect;[7,9] if, however, all N clones are represented in shape space by points outside v_0, then B cells will not recognize the antigen, with a potentially fatal result for the organism.[4,9]

Adopting this point of view one sees immediately that the probability that the representative point of a given B cell clone will be within v_0 will equal $\dfrac{v_0}{V}$, where V denotes the

total volume of that part of shape space that is immunologically relevant. Hence, the probability that this representative point will be located outside v_0 equals $1 - \frac{v_0}{V}$, and the probability that all N representative B-cell points are outside v_0 equals

$$\varepsilon \equiv proba bility\ of\ failure = \left(1 - \frac{v_0}{V}\right)^N \cong exp\left(-N\frac{v_0}{V}\right). \quad (9)$$

This estimate holds for a single infection. The probability of a successful immune response to this infection is then $1 - \varepsilon$, and the probability, P_s, that the organism will success-fully repel all cM infections during its lifespan is given by

$$P_s = (1 - \varepsilon)^{cM} \cong \exp(-\varepsilon cM). \quad (10)$$

Our design argument wants this probability to be very near to unity, so we require $\varepsilon\, cM \ll 1$ or

$$\varepsilon \equiv \exp\left(-N\frac{v_0}{V}\right) \ll \frac{1}{cM}. \quad (11)$$

For example, if $\varepsilon\, cM = 0.01$, then $P_s = 0.99$. Rewriting Equation 11 gives

$$N \gg \frac{V}{v_0} ln\ (cM), \quad (12)$$

which shows that N, the diversity of the B-cell repertoire, should scale $\sim ln\ (cM)$.

Combination of this result with the size $\sim M$ of each lymphocyte clone shows that the total number of B cells in the immune system should scale as $\sim M\, ln\ (cM)$. For example, the mouse probably has $\cong 10^7$ clones of B cells, with each clone containing $\cong 10$ cells.[10] What does this tell us about humans, whose body weight is about a factor 10^4 larger? First, because the size of each clone scales $\sim M$, the number of B cells per clone is expected to be $\cong 10^5$. Second, from Equation 12

$$\frac{N\,(human\,)}{N\,(mouse\,)} = \frac{ln\ cM\,(human\,)}{ln\ cM\,(mouse\,)} \quad (13)$$

in an obvious notation. Without knowing the value of the constant c we cannot make the statement more numerical. However, assuming arbitrarily that a mouse has to confront antigenic invasions $\cong 10$ times during its life, then cM (mouse) = 10, and

$$cM\,(human\,) = \frac{M\,(human\,)}{M\,(mouse\,)}cM\,(mouse\,) = 10^4 \cdot 10 = 10^5. \quad (14)$$

Hence from Equation 13 one finds N (human) $\cong 5N$ (mouse) $\cong 5 \times 10^7$, that is, a human should have a B-cell repertoire 5 times as large as a mouse. Further, if as argued in the preceding a human a clone contains 10^5 cells, then the total number of B cells in humans should be $\sim 5 \times 10^{12}$.

These estimates might be questioned because they depend on the somewhat arbitrary assumption that cM (mouse) = 10. However, the repertoire size in humans is only weakly dependent on the value assumed for cM (mouse). For example, the assumption cM (mouse) = 10^2 leads to a predicted human repertoire size of 3×10^7, cM (mouse) = 10^3 leads to a

human repertoire size of 2.3×10^7, and cM (mouse) = 10^4 gives a human repertoire of 2×10^7. Thus, the result that the human repertoire should not be much larger than the mouse repertoire seems fairly robust. This example also shows how we can use scaling relations to give insight into immune system design among different species.

Scaling the lymphatic vessels and the lymphocyte circulation time

The scaling relations which were derived in a rather heuristic way in the last two sections suggest some other scaling laws, providing one is willing to permit a slight level of speculation. As an example, we discuss the scaling of the lymphocyte circulation time, and of the tree of lymphatic capillaries.

A typical lymphocyte will continuously circulate through the body in a cyclical way. Each cycle consists of three stages:

1 The cell enters the circulation, and is carried by the blood to one of the body's service units.
2 It is released inside this service unit and diffuses around in it for a certain length of time, until it moves into a lym-phatic capillary.
3 It is transported through the lymphatic capillaries, vessels and lymph nodes, until it is inserted in the blood circula-tion again.

We ask for the typical times the cell spends in each of these three stages. For stage (1), it is obvious that this time is identical (in order of magnitude) to the circulation time of the blood, which was shown by West, Brown, and Enquist[2,3] to scale $\sim M^{1/4}$.

For stage (2), the typical time is (in order of magnitude) equal to the diffusion time, which was considered in the section 'Scaling of B and T cell clone sizes' and which scales $\sim M^{1/4}$ for the case of autonomous lymphocyte metabolism and $\sim M^{1/2}$ for the case in which lymphocyte diffusion follows the organisms metabolism.

In order to estimate the time that a lymphocyte spends in the lymphatic system, we note that this time will dominate the time spent in stages (1) and (2). Calling the time spent in stage (3), T_3, this means that the number of lymphocytes trans-ported per unit time scales $\sim M\, ln\ (cM)/T_3$. This quantity should be proportional to the amount of blood transported per unit time, because lymphocytes form a fixed fraction of the total amount of blood. The WBE theory[2,3] predicts that the total rate of blood transport scales $\sim M^{3/4}$. Hence one finds

$$M\, ln\ (cM)/T_3 \sim M^{3/4}, \quad (15)$$

which gives the predicted scaling relation

$$T_3 \sim M^{1/4}\, ln\ (cM), \quad (16)$$

for the time spent in the lymphatic system.

Equation 16 seems to suggest that the transport of a cell through the lymphatic system can be visualized as a sequence of $\sim M^\varepsilon\, ln\ (cM)$ steps, each of which requires an amount of time that scales as $M^{1/4-\varepsilon}$, where we expect ε to be small compared to 1. It is perhaps not too speculative to conjecture that each step consists of the passage of a cell through a specific lymph node, and that the number of lymph nodes through which a cell passes in its transit back to the blood

equals the number of steps. Hence, we conjecture the two scaling relations

$$\text{Time spent in a lymph node} \sim M^{1/4 - \varepsilon}, \quad (17)$$

$$\text{Number of lymph nodes traversed} \sim M^{\varepsilon} \, ln \, (cM) . \quad (18)$$

At this point, one can ask: how exactly does a lymphocyte pass through a lymph node? We are not aware of any detailed modelling of this transport process; hence, we suggest the following possibility. Suppose the incoming and outgoing lymphocytes are distributed throughout the volume, V_{LN}, of the lymph node by way of a system of transportation 'channels', and suppose this distribution system has the form of a self-similar, space filling, branching tree. As was shown in [2] and [3], this would imply that the total time for a cell to travel through the lymph node would scale $\sim V_{LN}^{1/4}$. As we found this time to scale $\sim M^{1/4-\varepsilon}$, the two results are consistent only if $V_{LN} \sim M^{1-4\varepsilon}$. Hence, since the density of the tissue is approximately 1, we find

$$\text{Mass of a lymph node} \sim M^{1-4\varepsilon} . \quad (19)$$

The total mass in a single string of lymph nodes, would follow from Equations 18 and 19

$$\text{Mass of a string of lymph nodes} \sim M^{1-3\varepsilon} \, ln \, (cM) . \quad (20)$$

This would be consistent with the scaling of the total number of B cells

$$\text{Total number of B cells} \sim M \, ln \, (cM) , \quad (21)$$

which, of course, follows from Equations 8 and 12 if the number of strings of lymph nodes scales as

$$\text{Number of strings of lymph nodes} \sim M^{3\varepsilon} . \quad (22)$$

In summary, we have derived nine scaling laws – Equations 8, 12 and 16–22 – all of which could in principle be verified (or falsified) by immunological experiments.

Miscellaneous remarks

The B cells, which formed the subject of the previous considerations, are only one of various cell populations that make up the immune system. This suggests that the scaling of these other types of cells can be derived from the scaling of the B cells provided enough is known about the pertinent biology. As an example, consider the T-cell population, which has a physiology similar to that of the B cells. Further, for a B cell to act it typically needs the collaboration of a T cell. This immediately suggests that T-cells scale in the same manner as B cells, that is, T cells clone sizes should scale $\sim M$, the number of T-cell clones should scale $\sim ln \, (cM)$ and the total number of T cells should scale $\sim M \, ln \, (cM)$. Recent estimates of the T-cell repertoire in mice and humans are in agreement with these predictions. Arstilla *et al.*[11] estimate the T-cell repertoire in humans is at least 2.5×10^7, while the T-cell repertoire in mouse spleen is about 2×10^6.[10] Since the

spleen in mice comprises approximately 20% of the T cells, the repertoire could be as much as five times larger, but based on these comparisons it is easy to suggest that with a 10^4-fold difference in mass, the repertoires of man and mouse differ only by less than a factor of 10, consistent with our predicted scaling relation.

An important issue that we have not explored is autoimmunity. Considerations of repertoire size are also impacted by the need of the immune system not to react to self.[12,13] Thus, issues such as the number of self antigens need to be considered and its relationship to body mass examined. Such issues are outside the scope of this paper but clearly warrant examination.

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