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Articles

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T Helper Cell Polarization and the Generation of Varied Antigen-dependent Immune Responses: A Markov Chain Model

Key Words. Lymphocyte, Markov chain, adaptive immunity, stochastic modeling, Th polarization.

Abstract. ***Background:*** *Adaptive immune responses are deterministically classified into humoral or cell-mediated depending on the pattern of Th cell polarization into Th1 or Th2. Evidence suggests that the process of Th polarization is stochastic, however, the presence of some deterministic components has not been ruled out. Here, a Markov chain model that accounts for Th-mediated immune responses was developed based on the assumption that Th polarization and consequent transition events are stochastic.* ***Results:*** *Using assumed probability values, model analysis suggests that there is a rapid convergence to produce an immune response once the Th cell is stimulated by an antigen which is amplified as the number of transitions increase. The expected number of visits between Th and itself, B and itself and Tc and itself is about one whereas it is zero, less than one or ∞ in the rest of the transition events depending on the interacting states.* ***Conclusions:*** *Based on model analysis and validation, modeling Th-mediated immune responses as a Markov chain process seems to be plausible. The large degree of flexiblity*

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inherent in such a view of adaptive immunity can be helpful in addressing questions pertinent to Th function and behavior.

1. BACKGROUND

T helper (Th) lymphocytes play the central role in the activation and clonal expansion of effector immune cells through an extensive family of interleukins (ILs) or cytokines (Mosmann *et al.* [1986]; Mosmann and Coffman [1989]). Based on the profile of cytokine production, Th cells are conventionally grouped into two subsets; Th1 and Th2 (Mosmann and Coffman [1989]; Abbas *et al.* [1996]; Romagnani [1994]). Th1 cells produce several cytokines including interferon-γ (INF-γ), granulocyte monocyte colony stimulating factor (GM-CSF), tumor necrosis factor (TNF-α), IL-2 and IL-3. Th2 cells produce cytokines like IL-3, IL-4, IL-5 and IL-10 (Mosmann *et al.* [1986]; Mosmann and Coffman [1989]; Abbas *et al.* [1996]; Romagnani [1994]). Understandably, Th0 cell polarization to Th1 or Th2 has great bearing on the kind of immune responses generated against a particular antigen. Th1 cytokines target and help stimulate $CD8^+$ cytotoxic T lymphocytes, natural killer (NK) cells and macrophages to kill infected cells while those produced by Th2 activate B cells to produce antibodies and enhance several innate immune responses (Mantovani [1999]; Swain [1999]; De Carli [1994]; Romagnani [1999]). New evidence suggests that the Th1/Th2 dichotomy in mammalian systems is more relative than absolute (Cohen [2000]; Fishman and Perlson [1994]; Fishman and Perlson [1999]; Bergmann *et al.* [2001]). Thus, the distinction between Th2-mediated humoral responses and Th1-mediated cellular responses is not clear-cut. Furthermore, the spectrum of cytokine profile-based classification of Th subsets is broadening as new cytokines and new Th subsets like Th3, Th4 and Th17 are being described (Mosmann and Sad [1996]; Tato *et al.* [2006]; Weaver and Murphy [2007]; Murphy [2005]). However, how new Th subsets fit into the Th-mediated immune response scheme is still ambiguous.

Naïve Th cells (Th0) have the potential to differentiate to either Th1 or Th2 (Mosmann and Coffman [1989], Abbas *et al.* [1996];

Romagnani [1994]; Sad and Mosmann [1994]; Swain *et al.* [1996]) or to other subsets such as Th17, which has been implicated in the exacerbation of autoimmune pathologies (Tato *et al.* [2006]; Weaver and Murphy [2007]; Murphy [2005]; Bettelli and Kuchroo [2005]). Interestingly, Th17 development depends on TGF-beta, which is also linked to regulatory T cell development and function, perhaps suggestive of a unique mechanism for matching Th cell effector and regulatory lineage specification (Weaver *et al.* [2006]). Evidence suggests that switches between polarized Th states do occur (Fishman and Perlson [1994]; Fishman and Perlson [1999]; Seder and Prussin [1997]; Coffman *et al.* [1995]; Yates *et al.* [2000]). Many of the factors which act on naïve $CD4^+$ Th cells to differentiate into either state has been explored (Fishman and Perlson [1994], Yates *et al.* [2000]; Viola and Rao [1999]; Tylor-Robinson *et al.* [1994]; Dong and Flavell [2000]; Miyatake *et al.* [2000]; Ismail and Bretchscher [2001]; Corthay [2006]); however, the exact combination of factors that favor the appearance of Th1 or Th2 are still debatable. Therefore, whether the process of Th polarization and the consequent events thereof are predetermined or stochastic is yet to be established. Recently, a hybrid model that accommodates both stochastic and deterministic processes to simulate the dynamic behavior of selective versus instructive Th-cell development was described. The model yielded close qualitative agreement with a number of well-established experimental observations (Jansson *et al.* [2006]). However, besides the fact that data reported was inconclusive to say the least, the model fell short of differentiating between the predetermined and stochastic components of Th differentiation.

Evidence suggests that IL-4, acting through stat-6, activates the transcription factor GATA3 therefore promoting Th0 polarization to Th2. IL-4 also blocks the transcription factor T-bet therefore inhibiting the generation of Th1 type cells (Weaver and Murphy [2007]; Murphy [2005]; Matsuoka *et al.* [2004]; Coccia *et al.* [2005]; Monteleone *et al.* [2004]; Salvati *et al.* [2005]; Afkarian *et al.* [2002]; Lit *et al.* [2007]). IL-12 on the other hand directs Th0 polarization to Th1 while blocking the generation of Th2. In this respect, IFN-γ, acting through stat-1, activates the transcription factor T-bet therefore promoting Th0 polarization to Th1. Con-

currently, IFN-γ blocks GATA-3 therefore inhibiting the generation of Th2 type cells. Despite these recent discoveries, several pertinent questions still linger. For example, how can one explain the generation of concurrent Th1- and Th2-mediated responses against the same infection? What determines the preliminary upregulation of IL-4 versus IL-12/IFN-γ that differentially directs the process of polarization one way or the other? Furthermore, it is yet to be determined whether the disparate actions of IL-4 versus IL-12/IFN-γ target uncommitted Th cells or whether they differentially select for Th1 versus Th2 from a preexisting and already committed Th cell pool.

In this study, we attempted to develop a stochastic model based on Markov chain theory to simulate the function of Th as it mediates the activation and clonal expansion of effector immune components based on the assumption that the behavior of Th differentiation is stochastic (Swain [1999]; Fishman and Perlson [1999]; Jansson *et al.* [2006]; Bar-Or [2000]). Stochastic modeling of various aspects of adaptive immunity using Markov chain theory is common in theoretical immunology. Modeling of Th-mediated immune responses using Markov chain theory has also been previously attempted though in a different context (Rarick *et al.* [2006]; Yates *et al.* [2001]). Markov chain processes represent one of the largest and most important areas of the theory of stochastic processes evidenced by the fact that it has found many applications in biology (Elkarmi *et al.* [1988]; Elkarmi and Dia [1993]; Elkarmi and Karmi [1998]; Davidson [1998]; Burke *et al.* [1997]; McGilchrist *et al.* [1989]; Rogers *et al.* [1998]; Tuckwell and Le Corfec [1998]). The choice to use Markov chain theory instead of more conventional stochastic processes (Bergmann *et al.* [2001]; Yates *et al.* [2000]) to model Th polarization better satisfies the premise that the behavior of Th-mediated immune responses can be viewed as a number of states or compartments. The recent findings reported by Rarick and coworkers (Rarick *et al.* [2006]) regarding the use of Markov chain theory to model the accumulation of Th1- and Th2-produced proteins (IL-2, IL-4, IL-8, IL-10, IL-12, IFN-γ, TNF-α and MCP-1) during gonococcal infections is further support to the validity of choosing a Markov chain modeling strategy in this study. Furthermore, dependence between

the successive events that lead to Th0 polarization suggests a behavior more compatible with Markov chain theory than with the classical theory of statistics, which mainly deals with independent yet identically distributed events.

Using arbitrary transition probabilities, computer-based model analysis suggests that a rapid convergence to produce an immune response ensues following Th cell/stimulus (antigenic peptides in the context of self major histocompatibility complex class II molecules) engagement. The response seems to be further amplified as the number of transitions increase. The expected number of visits between Th and itself, B and itself and Tc and itself is around one whereas it is 0, <1 or ∞ in the rest of the transition events.

2. MODEL FORMULATION AND MODEL ASSUMPTIONS

A Markov chain is a sequence of random variables such that for any n, X_{n+1} is conditionally independent of X_0 …, X_{n-1} given X_n. That is, the "next" state X_{n+1} of the process is independent of the "past" states X_0…, X_{n-1} provided that the present state X_n be known. In other words, Th cell polarization into a cytotoxic T lymphocyte is dependent on the event that occur at the present state (Th) but independent of all other previous events (states). Thus, the behavior of the immune system from the onset of T helper function to the production of an immune response is assumed to be a stochastic process which can be modeled as a Markov chain with a state space of four states; S = [Th, Tc, B, R].

Th: T helper cell which can differentiate into Th1 or Th2 where each of these two states can mediate a disparate set of consequent immune responses.

Tc: T cytotoxic cell (along with NK cells and macrophages)

B: B cells that generate antibodies as mediators of different effector functions

R: the response (this refers to humoral responses generated by B cells or cell-mediated responses generated by T cytotoxic cells, macrophages, dendritic cells… etc.).

These four states represent the actual humoral or cell-mediated immune responses when elicited by an antigen where Th cells may

activate a B cell or a cytotoxic T cell or both. The actual stages of the immune response are transformed to a chain of variables (states), which have a Markov property. The movement in the process between the states is such that the Th cell undergoes transitions between Th, Tc and B states. Transition in this context refers to the relevant transition probabilities (as explained below); it should not therefore be taken to mean the actual transformation of one cell type to another. A transition probability reflects the probability that a Th cell will differentiate into a Th1 or Th. The B state undergoes transitions between Th, B and R while the Tc state undergoes transitions between Tc and R, an assumption which accounts for the capacity of B, but not Tc, cells to function as antigen presenting cells (APCs) to Th cells. Model formulation takes into account that only B cells and T cytotoxic cells (as well as other effector cells) can produce a response but not Th cells. A transition graph incorporating the four states of the model where a line from one state to the next means a positive probability is depicted in figure 1. Implicit in figure 1 is the assumption that Th randomly polarizes into a state that favors the mediation of humoral or cell-mediated effector functions. In other words, no distinct Th1 or Th2 state appears in the figure based on recent evidence which suggest that such a distinction is more apparent than real (Cohen [2000]; Fishman and Perlson [1994]; Fishman and Perlson [1999]; Bergmann *et al.* [2001]). In doing so, we believe that the appearance of new Th subsets can be easily accommodated into the model; the Th2-like Th17 subset is a case in point. As shown in figure 1, the transition probabilities were assumed such that the possibility that Th can activate B, Tc, neither or both can be accounted for. P_1 is the probability of Th remaining in the Th state, i.e., the probability of producing a memory Th cell. P_2 is the probability of Th polarizing into Th2 to activate B cells. P_3 is the probability of activating a Tc by Th (polarization to Th2). P_4 is the probability of a B cell remaining as a B cell without being transformed into R; this is important for the generation of memory B cells. P_5 is the probability of a Tc remaining as a Tc so as to produce memory Tc cells. P_6 is the probability of producing a response from B cells, i.e., the differentiation and activation of B cells into plasma cells and the subsequent production of anti-

bodies as effectors. P_7 is the probability of producing a response from Tc cells, i.e., the differentiation and activation of Tc cells into active cytotoxic T cells that kill infected cells. P_8 is the probability of activating Th by B cells mainly when B cells function as APCs to Th cells (mainly applies to secondary immune responses). It is worth noting that in a Markov chain, the main emphasis is on the chance of moving from one state to another in a fixed number of steps or the number of times the process visits a certain state. No consideration is given in the model to the actual time spent in between the transitions, which is quite suitable for the purpose intended here.

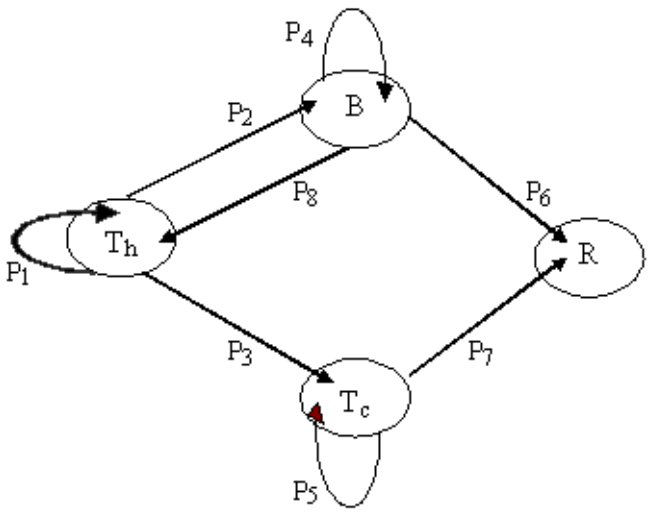

Figure 1 – Transition graph incorporating the four states of the stochastic model developed in this study. The details of Th polarization into different Th subsets are excluded from the figure as the basic assumption implies relativity and randomness.

The following assumptions were considered in formulating the model:

1. Various aspects of adptive immunity can be modeled as a Markov chain (Rarick *et al.* [2006]; Yates *et al.* [2001]); it is counter-intuitive to envision Th polarization as predetermined because the same antigen can induce the polarization of Th cells to become Th1, Th2 or both depending on the Th0 priming sig-

nals. Should Th polarization be a predetermined process, the degree of flexibility or plasticity of adaptive immunity will no doubt be minimal, which is in direct opposition to current understanding of Th-mediated immune responses. Furthermore, neither the Th1/Th2 dichotomy nor the sequence of steps that follow Th differentiation are absolute.

2. The process starts by the activation of Th cells by complex interactions with antigen presenting cells (APC); thus it represents the response of the immune system to T-dependent antigens. Th in this model includes both Th1, Th2 and any number of operative Th subsets already known to exist or yet to be described.
3. The process involves the participation of B cells, Tc cells or both. This is dependent on the inputed initial transition probabilities.
4. The process ends by the production of a response by B cells in the form of plasma cells producing antibodies, by cytotoxic effector function of Tc, both or niether. Absence of a response may entail generation of immunologic memory, death of target cells or tolerance induction if the response is to be quantified by the degree of antigen elimination/persistence. Though not within the scope of this study, the model can be modified so as to distinguish between the various possible forms of the R state. The probability of producing no response from B or Tc cells is represented in the model by a transition probability equal to zero.
5. The role of Tc, macrophages and other types of cells as cytokine producers influencing the various transition states are ignored. Also ignored are the possibilities that Th cells may function as killer cells and that immune responses other than cell-mediated or humoral are generated following Th cytokine-mediated activation.

3. MODEL ANALYSIS

The stochastic process $X = \{X_n; n \in N\}$ is called a Markov chain provided that:

$$P\{X_{n+1} = j / X_0, \ldots, X_n\} = P\{X_{n+1} = j / X_n\}$$

For all $j \in S = [Th, B, Tc, R]$ and $n \in N = \{0, 1, \ldots\ldots\}$.

In other words,

$$P\{X_{n+1} = j / Xn = i\} = P\{i, j\}, i, j \in S,$$

where P {i, j} are the transition probabilities for the Markov chain. The transition matrix of the Markov chain $X = \{X_n ; n \in N\}$ and state space S = [Th, B, Tc, R] is:

$$P = \begin{array}{c} \\ Th \\ B \\ Tc \\ R \end{array} \begin{array}{c} \begin{array}{cccc} Th & B & Tc & R \end{array} \\ \begin{bmatrix} P_1 & P_2 & P_3 & 0 \\ P_8 & P_4 & 0 & P_6 \\ 0 & 0 & P_5 & P_7 \\ 0 & 0 & 0 & 1 \end{bmatrix} \end{array}$$

The probability that the chain moves from state i to state j in m steps equals the {i, j} entry of the m^{th} power of the transition matrix P. In other words, for any m ε N,

$$P\{X_{n+m} = j / X_n = i\} = P^m \{i, j\} \text{ for all } i, j \varepsilon S \text{ and } n \varepsilon N.$$

A state j is called recurrent if $P_j \{T < \infty\} = 1$; otherwise, if $P_j \{T = + \infty\} > 0$, then state j is called transient. Furthermore, a recurrent state j is called null if $Sj [T] = \infty$ where T is the time of first visit to state j; otherwise, it is called non-null. Thus, state (R) is non-null recurrent state and states (Th, Tc, B) are transient states. State (R) is an absorbing state. R (i, j) is defined as the expected number of visits to state j starting at state i. For a recurrent state $R (i, j) = \infty$ and if state j can be reached from state i, then $R (i, j) = \infty$. On the other hand, if state j can not be reached from i, then R (i, j) = 0.

For transient state R (i, j) can be calculated as follows.

Let C denote the set of all transient states and let Q and E be the matrices obtained from P and R respectively by deleting all the rows and columns corresponding to the recurrent state. In other

words, Q (i, j) = P (i, j) and E (i, j) = R (i, j), i, j ∈ C. Therefore, it can be shown that:

$$E = \sum Q^m = I + Q + Q^2 + \ldots, \text{ and } E = (I - Q)^{-1}. \text{ Thus,}$$

$$Q = \begin{bmatrix} P_1 & P_2 & P_3 \\ P_8 & P_4 & 0 \\ 0 & 0 & P_5 \end{bmatrix} \tag{1}$$

and

$$E = \begin{bmatrix} 1 - P_1 & -P_2 & -P_3 \\ -P_8 & 1 - P_4 & 0 \\ 0 & 0 & 1 - P_5 \end{bmatrix}^{-1} \tag{2}$$

If the determinant of (I – Q) denoted by $|I - Q| \neq 0$, then the matrix (I – Q) is nonsingular and has an inverse. From the definition of a Markov chain, $\sum_j P(i, j) = 1$ for each i, j ∈ S. Therefore,

$$P_1 + P_2 + P_3 = 1 \text{ or } P_1 = 1 - (P_2 + P_3) \tag{3}$$

and

$$P_8 + P_4 + P_6 = 1 \text{ or } P_4 = 1 - (P_8 + P_6). \tag{4}$$

The determinant of (I – Q) is calculated to be $(P_3P_8 + P_2P_6 + P_3P_6)(1 - P_5)$. Since all these probabilities are positive, then $|I - Q| > 0$ and the matrix (I – Q) is nonsingular and has an inverse. The matrix $(I - Q)^{-1}$ is calculated to be:

$$E = \frac{1}{P_9P_7} \begin{bmatrix} (1 - P_4)(1 - P_5) & P_2(1 - P_5) & P_3(1 - P_4) \\ P_8(1 - P_5) & (1 - P_1)(1 - P_5) & P_3P_8 \\ 0 & 0 & PP \end{bmatrix}^{-1} \tag{5}$$

When $PP = (1 - P_1)(1 - P_4) - P_2P_8$ and

$P_7 = 1 - P_5$ and let $P_9 = (P_3P_8 + P_2P_6 + P_3P_6)$, then

$$E = \begin{bmatrix} \frac{(1-P_4)}{P_9} & \frac{P_2}{P_9} & \frac{P_3(1-P_4)}{P_9P_7} \\ \frac{P_8}{P_9} & \frac{(1-P_1)}{P_9} & \frac{P_3P_8}{P_9P_7} \\ 0 & 0 & \frac{PP}{P_9P_7} \end{bmatrix} \quad (6)$$

Therefore, the R (i, j) usually called the potential matrix after replacing the recurrent state first becomes:

$$R = \begin{array}{c} \\ R \\ Th \\ B \\ Tc \end{array} \begin{array}{c} \begin{array}{cccc} R & Th & B & Tc \end{array} \\ \begin{bmatrix} \infty & 0 & 0 & 0 \\ 0 & \frac{(1-P_4)}{P_9} & \frac{P2}{P_9} & \frac{P_3(1-P_4)}{P_9P_7} \\ \infty & \frac{P_8}{P_9} & \frac{(1-P_1)}{P_9} & \frac{P_3\,P_8}{P_9P_7} \\ \infty & 0 & 0 & \frac{PP}{P_9P_7} \end{bmatrix} \end{array} \quad (7)$$

Transition probabilities and R (i, j) values were calculated using a computer program specially designed for this purpose. The input of initial probabilities required for the calculations were assumed to arbitrarily mimic the dynamics of Th-mediated immune responses. It is possible to use any number of other combinations of initial probabilities to test and validate the model but all such combinations, like the ones used here, would have to be assumed for lack of actual data. The initial probabilities used were as follows:

$$P_{\text{initial}} = \begin{bmatrix} 0.05 & 0.475 & 0.475 & 0 \\ 0.05 & 0.1 & 0 & 0.85 \\ 0 & 0 & 0.1 & 0.9 \\ 0 & 0 & 0 & 1 \end{bmatrix}$$

4. RESULTS AND DISCUSSION

The transition probability function and the expected number of visits to a given state [R (i, j)] were calculated based on current understanding of the behavior of adaptive immunity. Table 1 shows the results of computer-based analysis of the transition probabilities for m = 2, 3, 4. As shown in table 1, there is a rapid convergence to produce a response once Th is stimulated by an antigen. This is indicated by the fact that all other probabilities are fast

Table 1 – Transition probabilities after two steps (m = 2), three steps (m = 3) and four steps (m = 4).

Transition probabilities after two steps:

	Th	B	Tc	R
Th	0.004875	0.019594	0.019594	0.955938
B	0.002063	0.006938	0.005938	0.985063
Tc	0	0	0.001	0.999
R	0	0	0	1

Transition probabilities after three steps:

	Th	**B**	**Tc**	**R**
Th	0.001224	0.004275	0.004275	0.990227
B	0.00045	0.001674	0.001574	0.996303
Tc	0	0	0.0001	0.9999
R	0	0	0	1

Transition probabilities after four steps:

	Th	**B**	**Tc**	**R**
Th	0.000275	0.001009	0.001009	0.997708
B	0.000106	0.000381	0.000371	0.999142
Tc	0	0	0.00001	0.99999
R	0	0	0	1

changing to a value close to zero except those that lead to a response. This rapid convergence occurs regardless of the values of the initial probabilities. This is in line with current immunologic thought; in that, a single Th-antigen interaction event usually leads either to a humoral or a cell-mediated response but not to both at the same time. However, a second Th-antigen interaction event may lead to a varied response. This may help explain the observation that a single antigenic epitope, let al.one the multitude of epitopes present in a whole pathogen, can often elicit concurrent humoral and T cell-mediated immune responses. Furthermore, according to the model, there is crosstalk between Th and B cells that initially favors the polarization of Th into Th2. The rapid convergence to a response increases as the number of transitions (m's) increases, vis-à-vis, the degree of crosstalk between a Th and B or Tc. This behavior, as stated earlier, occurs no matter what the initial probabilities are. For example, the reciprocal activation of B cells by Th cells and of Th cells by B cells to increase cytokine concentration leads to a higher probability of reaching a humoral immune response (Swain [1999]; Fishman and Perlson [1994]; Tylor-Robinson *et al.* [1994]).

The number of transitions can be viewed as the availability of the specific cytokine profile essential to polarize Th one way or the other. Assuming that this holds true, it would be possible to address many interesting questions pertaining to the combinatorial effects that direct Th polarization in either direction. It will be possible to evaluate the cytokine profile that leads to a specific response and the possibility of manipulating such a profile to switch the Th phenotype in experimental or therapeutic settings. Additionally, perturbed Th balance in autoimmunity (Tuckwell and Le Corfec [1998]; Touil *et al.* [2006]), pregnancy-related immunosuppression (Raghupathy [1997]), allergic asthma (Inoue *et al.* [2007]; Zhang-Hoover and Stein-Streilein [2007]), inflammatory bowel disease (Monteleone *et al.* [2006]), Behcet's disease (Suzuki *et al.* [2006]) and several other Th1/Th2 imbalance-exacerbated immunopathologies (Monteleone *et al.* [2006]; Tellides and Pober [2007]) can be more readily addressed.

Table 2 illustrates the results of computer-based model analysis for the expected number of visits to each of the four states. By

definition, the expected number of visits between R to R, Tc to R and B to R is ∞ while Th to R is zero as Th cells are not involved in effector functions. The expected number of visits between Th and itself, B and itself and Tc and itself is approximately one; this is essential if memory is to be generated. This finding closely resembles the actual dynamics of adaptive immunity in relation to the generation of immunologic memory following the first encounter with a foreign antigen. Furthermore, it suggests that varied "immunologic memories" can be concurrently generated by the same pathogen. In agreement with current understanding, the expected number of visits between Th and B or Tc is similar indicating that Th can transit into either state depending on the combination of factors that favor one state over the other (Fishman and Perlson [1994], Fishman and Perlson [1999]; Yates *et al.* [2000]; Viola and Rao [1999]; Tylor-Robinson *et al.* [1994]; Dong and Flavell [2000]). It is interesting to note that the expected number of visits between B and Th is twice as high as that between Tc and Th; in other words, there is more cross-talk between Th and B compared with that between Th and Tc. This may help explain the observation that the first polarization event, according to our model, usually favors the generation of a humoral immune response. At face value, this may suggest that the adaptive immune response is more inclined to develop as humoral rather than cell-mediated; however, the type of antigen presenting cells (APC) and/or the presence of Tc-specific cytokine profile may help the Th state to transit into a Tc state. If the expected number of visits between different states is viewed as, for example, the concentration of the cytokine profile (Mantovani [1999]; Swain [1999]; De

Table 2 – R(i, j) showing the expected number of visits to each state.

	R	**Th**	**B**	**Tc**
R	∞	0	0	0
Th	0	1.082707	0.571429	0.571429
B	∞	0.06015	1.142857	0.031746
Tc	∞	0	0	1.111111

Carli *et al.* [1994]; Fishman and Perlson [1994], Fishman and Perlson [1999]; Dong and Flavell [2000]), then the higher the cytokine concentration the more the number of visits will take place to the respective state. For example, generation of memory against a specific antigen at the Th level is dependent upon the occurrence of visits between Th and itself. In other words, antigens that induce increased synthesis of "memory-generating-cytokines" by Th may be more successful in generating memory compared with other types of antigens (Swain *et al.* [1996]).

Modeling the immune response in this fashion mimics real life, in that it considers the adaptive immune response as a number of stochastic Th/antigen interactions that lead to separate and possibly different consequences. It further translates each Th/antigen interaction event into distinct transition probabilities and hence a random outcome. Th/antigen interactions represent the essential first stochastic step for all specific immune responses to occur. But even if this critical step is passed, other steps or transition states in the adaptive immune response cascade (figure 1) may or may not follow depending on the transition probabilities of the preceding state. Therefore, all possible outcomes or scenarios that may occur upon antigen encounter can be accounted for. A specific antigen may induce a humoral immune response, a Tc immune response, memory, tolerance or cell death either separately or in various combinations. Additionally, qualitative differences between primary and secondary immune responses can be invisioned using this model. Viewing the progression of the immune response as described in this model allows for studying the immune response in health and disease by assigning different probabilities to the various steps. The model can account for T cell, B cell and antigen presenting cell defects and abnormalities. This is clearly illustrated in the assumption that R probability ranges from zero to one. It is worth stressing that the responses indicated by the model such as the rapid convergence to a response will occur no matter what the initial probabilities are. Therefore, conclusions reached by using this model are not greatly affected by changing the initial probabilities since the model will behave in the same way regardless. Other probabilities will just change how rapid the convergence event will be.

Notwithstanding the fact that linear deterministic modeling has been used in the past to model T helper function (Burke *et al.* [1997]), the attempt to use Markov chain modeling in the context of this study is well justified. The transition probabilities of the model determine the different probabilities of transition from one state to another while Markov chain determines the sequence and classification of the various transition state. Each transition probability in such a model is equivalent to the expected value of the corresponding rate coefficient (constant) of the linear model. Each coefficient indicates the rate of activation of one type of cell or the other and the rate of producing a response. On the other hand, the transition probability function indicates the different probabilities of transition, and thus determines when and how a response will be attained. Thus, Markov chain rather than linear deterministic modeling was used for several reasons: First, the validity of using linear deterministic modeling to simulate a process that is likely to be probabilistic is questionable. Without definite and complete evaluation of the process, common sense dictates that the simplifications of linear deterministic modeling ought not be made. Second, some of the probabilities in this model have no equals in linear modeling, thus linear deterministic modeling can not capture the true picture of Th figures in the immune system. Third, the use of Markov chain in the context of this study can be of value in explaining the immune system both in health and disease.

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POLARIZZAZIONE DELLA CELLULA T HELPER E GENERAZIONE DI DIVERSE RISPOSTE IMMUNITARIE ANTIGENE-DIPENDENTI: UN MODELLO A CATENA DI MARKOV

Riassunto

Le risposte immunitarie adattative sono deterministicamente classificate in "umorali" o "mediate da cellule" a seconda del pattern di polarizzazione della cellula T helper (Th) in Th1 o Th2. Le evidenze sperimentali suggeriscono che il processo di polarizzazione sia di natura stocastica, anche se la presenza di alcune componenti deterministiche non può essere esclusa. In qesto lavoro viene sviluppato un modello a catena di Markov per le risposte immunitarie Th-mediate, basato sull'assunzione che la polarizzazione della cellula Th e i conseguenti eventi di transizione siano stocastici.