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Neonatal Tolerance Revisited by Mathematical Modelling

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The classical view of a neonatal tolerance window has recently been challenged by several studies showing that neonates can evoke normal immune responses. For example, neonatal immunity against the male antigen H-Y can be induced in female recipients by inoculation of male donor spleen cells enriched for professional antigen-presenting cells (APCs). In the same set-up, adult female recipients become tolerant by giving large doses of spleen cells. Using a probabilistic model, we here show how the number of T cells and endogenous APCs in the recipient, and the dose and quality of donor APCs can explain all observed phenomena. We thus reconcile the classical neonatal tolerance window with the recent data on neonatal immunity and adult tolerance.

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There is increasing evidence that antigenic stimulation during the classical tolerance window of prenatal or neonatal life [1, 2] can evoke normal immune responses [3–6]. The seminal work of Ridge and co-workers [3] showed that inoculation of female mice with syngeneic male spleen cells could evoke both immunity and tolerance to the male antigen H-Y at any age of the mice. The two key factors determining the end result were the fraction of professional antigen-presenting cells (APCs), i.e. male dendritic cells, in the inoculum, and the dose of spleen cells given. If neonates were injected with highly enriched male dendritic cells expressing costimulatory molecules (i.e. signal two [7]), an immune reaction was evoked. Because a similar mechanism holds for adult naive T cells, these results were taken to contradict the classical view of a neonatal tolerance window. Additionally, it was shown that adult mice, having more T cells, could be rendered tolerant to the H-Y antigen if a high dose of spleen cells was given. On average, tolerance induction required five spleen cells per resident T cell [3].

Following the original argumentation [3], a simple mathematical model is developed here, which fully explains these results in terms of the probability that a naive T cell encounters a professional APC. The model reconciles the recent experiments [3] with the classical views that during early life tolerance is induced [1], which might be caused by a defect in antigen presentation [8, 9].

Questions answered by the model are: why would tolerance induction

(1) be more difficult in older mice having a greater number of T cells [3],

(2) be facilitated by increasing the inoculum size [2, 3] and

(3) require a fixed ratio of five male spleen cells per resident female T cell [3]?

Thus, the model provides a mechanism for the classical tolerance window in terms of the number of antigen-specific T cells, n, and their probability of meeting a professional APC, α.

First, consider the effect of the number of T cells to be tolerized. The data (see Table 1) show that T-cell numbers increase with age, and that tolerance induction requires larger inocula in older mice (column 4). Simple Poisson statistics explain why T-cell numbers matter: having n antigen-specific T cells and a probability α that a T cell meets a professional APC, one expects αn T cells to become immunized, and $(1-\alpha)n$ T cells to become tolerized. Because the successful priming of only a few specific T cells is expected to prevent the induction of tolerance [3], it is required that virtually all naive specific T cells are turned off, i.e. it is required that $\alpha n < 1$ cell. Thus, the ease with which tolerance is induced decreases as the number of antigen-specific T cells increases, just because the expected number of primed T cells remains proportional to n.

The same calculation also explains why tolerance induction is more stringent in the classical tolerance experiments [1]. These classical experiments, being based upon an allogeneic system, should involve many more antigen-specific T cells, n. Hence, the tolerance window is indeed expected to close early, i.e. at a time when total T-cell numbers are still low. Additionally, by the same Poisson distribution one may expect some of the mice to become tolerant and some immune. This is exactly what is reported in the classical experiments [1, 2]. The experimentalists,

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Table 1. Experimental data from [3] and predictions derived from equation (1)

Age	Total T cells	Specific T cells	Inoculum in [3]	Ratio in [3]	Predicted inoculum	Predicted ratio
1 day	1.0×10^4	0.1	5.0×10^6	500	0	–
7 days	1.2×10^6	12	1.0×10^7	8.3	4×10^6	3.3
18 days	7.0×10^6	70	2.5×10^7	3.6	2.7×10^7	3.9
24 days	1.1×10^7	110	4.0×10^7	3.6	4.4×10^7	4.0
7 weeks	2.2×10^7	220	1.0×10^8	4.5		

Column one is the age of the female recipients, column two shows the total number of $CD4^+$ and/or $CD8^+$ splenic T cells as calculated from Fig. 1(B) in [3] (using linear interpolation for days 18 and 24), column three is the corresponding estimate for the number of H-Y-specific T cells for a precursor frequency of 1 per 10^5, column four is the reported minimal inoculum size for tolerance induction [3], column five is the ratio between columns four and two, column six is the predicted minimal inoculum size for tolerance induction as calculated from equation (1) and column seven is the ratio between columns six and two. The model predictions in columns six and seven were made for $f=0.001$ and $e=3.6\times10^5$. These predictions are fairly insensitive to this particular parameter choice, however. For example, lowering f 10-fold (and recalculating by equation (1) that $e=4.5\times10^5$), the predicted ratios in column seven remain close to five.

blaming this on their failure to successfully inject all of the fetuses [1], may thus have been too modest.

This argumentation leaves unexplained, however, how raising the inoculum size can facilitate tolerance induction: in small and large inocula one expects the same fraction of professional APCs. Hence the probability of meeting a professional APC is a priori not expected to depend on the inoculum size.

Endogenous professional APCs are also expected to present the male antigen [10]. We propose therefore that increasing the donor inoculum size works by giving higher numbers of non-professional APCs which 'dilute' endogenous professional APCs in the recipient. Mathematically, one computes the probability of encountering any professional APC by:

$$\alpha = \frac{e + fm}{e + m} \qquad (1)$$

where e is the number of endogenous professional APCs, f is the fraction of professional APCs in the inoculum and m is the inoculum size.

We only consider *professional* endogenous APCs because non-professional APCs are not expected to pick up and present the male antigen. Thus, increasing the inoculum size decreases α, and hence decreases the expected number of professionally stimulated T cells αn. Therefore, increasing the inoculum size may facilitate tolerance induction.

Finally, to check if the model results are consistent with the experimental data, we estimate the parameters of the model from the data of the 7-week-old mice. This allows us to calculate the ratios of male donor cells per resident female T cell that are theoretically required to induce tolerance. The original data [3] provide the total numbers of $CD4^+$ and/or $CD8^+$ splenic T cells (see Table 1, column 2). Assuming a precursor frequency for the male antigen of order magnitude 1 per 10^5 T cells, the number of antigen-specific T cells, n, is estimated in the third column. Seven-week-old mice, in which tolerance is induced at an inoculum size of order magnitude $m=10^8$ male spleen cells, are thus estimated to have $n=220$ H-Y-specific splenic T cells. Thus, for H-Y tolerance in 7-week-old mice it is required that the probability of meeting a professional APC α is smaller than 1 in 220 (because it is required that $\alpha n<1$). The fraction of professional APCs f in the donor inoculum should thus be lower than 1 in 220, i.e. 0.0045. Picking $f=0.001$ as an example, the 7-week-old mice should have $e\approx3.6\times10^5$ endogenous APCs (by equation (1) above). A fraction of 1 per 1000 professional APCs in the inoculum might seem low, but note that we only consider effective professional APCs homing into the appropriate lymphoid tissue.

Assuming, for the moment, that the number of endogenous APCs, e, remains fairly constant during the first weeks of life, and assuming $f=0.001$, the minimally required inoculum size, m, during the neonatal period can be calculated from equation (1) (see column 6 in Table 1).

Except for the newborn mice, our predictions fit the data well. The underestimation of the inoculum size required in newborn mice could be a result of the rapidly increasing number of T cells in these mice (i.e. 30-fold in 3 days [3]): these newly arising T cells would indeed require a higher inoculum size. The ratios between the required inoculum sizes and the total T-cell numbers in the data, and in the model, are given in columns five and seven, respectively. The original claim that tolerance is obtained when this ratio is approximately five [3] is indeed reflected in column five. The original interpretation of this fixed ratio was that in older mice larger inocula are required to 'overwhelm' the larger numbers of resident T cells. As we found similar fixed ratios (column 7), the model provides a more mechanistic interpretation for the 'overwhelming' of T cells. Larger inocula are required in older mice to further dilute the endogenous professional APCs to prevent them from stimulating any of the resident female T cells.

An interesting complication of the model would be to allow the numbers and/or quality of endogenous APCs to increase with age [8, 9]. In our model this would increase α, which would

provide a second explanation of why tolerance induction is more difficult in older mice.

Summarizing, the model suggests two mechanisms for the closing of the tolerance window during fetal or neonatal life. It is the combination of increased numbers of T cells [3] and functional professional APCs [8, 9], which closes the tolerance window at a critical age. Thus, the experiments in which T-cell numbers and the numbers of professional or non-professional APCs are varied [3], are in full agreement with our model for the closing of the classical [1] tolerance window.

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REFERENCES

1 Billingham RE, Brent L, Medawar PB. 'Actively acquired tolerance' of foreign cells. Nature 1953;172:603–6.
2 Billingham RE, Silvers WK. Studies on tolerance of the Y chromosome antigen in mice. J Immunol 1960;85:14–26.
3 Ridge JP, Fuchs EJ, Matzinger P. Neonatal tolerance revisited: turning on newborn T cells with dendritic cells. Science 1996;271:1723–6.
4 Sarzotti M, Robbins DS, Hoffman PM. Induction of protective CTL responses in newborn mice by a murine retrovirus. Science 1996;271:1726–8.
5 Forsthuber T, Yip HC, Lehmann PV. Induction of TH1 and TH2 immunity in neonatal mice. Science 1996;271:1728–30.
6 Aguirre KM, Garvy BA, Johnson LL. Acquired resistance to *Cryptococcus neoformans* in adult mice vaccinated as newborns. Infect Immun 1997;65:1688–94.
7 Bretscher P, Cohn M. A theory of self-nonself discrimination. Science 1970;169:1042–9.
8 Lu CY, Calamai EG, Unanue ER. A defect in the antigen-presenting function of macrophages from neonatal mice. Nature 1979;282:327–9.
9 Lu CY, Beller DI, Unanue ER. During ontogeny, Ia-bearing accessory cells are found early in the thymus but late in the spleen. Proc Natl Acad Sci USA 1980;77:1597–601.
10 Geginat G, Gunther E. Genetic control of the cellular *in vitro* response to the H-Y antigen in the rat. Transplantation 1993;56:448–52.