Mathematical Model of a Virus-neutralizing Immunoglobulin Response

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We present a mathematical model to simulate the kinetics of B-cell activation and the virus-neutralizing immunoglobulin response in the spleen of mice after infection with vesicular stomatitis virus (VSV). Our model combines data from in vitro experiments and in vivo kinetic observations. A system of eight nonlinear differential equations was used in the computer experiments and numerically solved. The isotype switch from IgM to IgG in the presence ofirgin T-cell help was modelled by a time variable function, used as a parameter.

The model solutions indicate fast kinetics of the generation of VSV-neutralizing IgM antibodies within 2–3 days post immunization peaking on day 5 at a serum concentration of ~ 80 µg ml⁻¹ IgM, which is equivalent to about 10% of the total IgM serum concentration. The frequency of virus-specific B cells increases about 1000-fold within the first 4 days after immunization. Protective levels of VSV-neutralizing IgG antibodies (≥ 10 µg ml⁻¹) are reached within 5 to 6 days post immunization. Fitting the model solutions to the experimentally observed neutralizing serum titers suggests an increase in the neutralizing activity of IgGs occurring between days 5 and 8 post-infection. The model indicates that less than 10 VSV-specific B cells have to be triggered daily to maintain protective IgG serum titers during the memory phase.

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1. Introduction

Vesicular stomatitis virus (VSV) is a highly cytopathogenic negative-strand RNA rhabdovirus closely related to rabies virus. VSV replicates only in neuronal tissues of mice whereas in gene knock-out mice lacking a functional interferon system the virus replicates in virtually all tissues (Steinhoff et al., 1995; Kalinke et al., 1996b). Neutralizing antibodies are necessary and sufficient for protection against lethal infection with VSV. The surface envelope of VSV contains ~1200 identical G-protein molecules which form a regularly and densely ordered quasicrystalline structure (Wagner, 1987). On the G-protein molecule of live virus one major antigenic site is accessible for binding by neutralizing antibodies. Neutralization of VSV is mediated by covering 30–50% of the G-protein epitopes by neutralizing antibodies which either block docking of VSV to target cell receptors or inhibit fusion or pH-dependent conformational changes in the endosomes (Flamand et al., 1993; Kalinke et al., 1996b; Bachmann et al., 1997). Within the first hours after experimental VSV infection of mice, virus spread is limited by the interferon system.
(Steinhoff et al., 1995; Kalinke et al., 1996b). In the early phase of the immune response virus specific precursor B cells are stimulated by binding of presumably very limited numbers of viral particles to the antigen-specific receptors of B cells which leads to extensive B cell receptor crosslinking. Stimulated B cells undergo clonal expansion and differentiate into antibody forming cells (AFCs) which secrete VSV-neutralizing antibodies of the M isotype (IgM). Between day 4 and 7 in the presence of T-cell help such AFCs may switch the expression of antibodies of the M isotype (IgM) to the expression of antibodies with the same specificity of the G isotype (IgG) (Andersson et al., 1978; Leist et al., 1987; Bachmann et al., 1994; Kalinke et al., 1996a). In the memory phase, VSV-neutralizing IgG antibody titers are detectable in the serum for life (Bachmann et al., 1994).

The numbers of AFCs required to produce the neutralizing IgG antibody titers observed during the early phase of the VSV-neutralizing immunoglobulin response by day 8 was 10 to 20 times higher than the numbers of AFCs required to reach the same antibody titers in the late phase of the immune response (> day 21). This observation required explanation which was provided by a mathematical model (Bachmann et al., 1994). The model consisted of two independent sets of equations describing the early phase and the memory phase of the immune response, respectively. The early phase is characterized by an exponential growth of AFCs [eqn (1)], which have a cell doubling time of 7–8 hr resulting in an AFC growth rate constant ($\lambda$) of about 2.1 per day, and an exponential growth rate of the IgG serum concentration produced by the AFCs [eqn (2), first term]. The antibody production rate constant ($p$) is $10^5$ IgG molecules per cell and day. At the same time the IgG half-life in the serum of mice is about 1 week resulting in an IgG decay rate constant ($\mu$) of about 0.1 per day ($\mu = \ln(2)t_{1/2}$).

Antibody forming cells $\frac{dAFC}{dt} = \lambda \cdot AFC$  

IgG antibodies $\frac{dAb}{dt} = p \cdot AFC - \mu \cdot Ab$  

Equation (1) has the solution $AFC(t) = AFC(0) \cdot e^{\lambda t}$, whereas the solution of eqn (2) after insertion of the solution of (1) is 

$$Ab(t) = \frac{p}{\lambda + \mu} \cdot AFC(t) \cdot (e^{\mu t} - e^{-\mu t})$$

Since the AFC growth rate constant ($\lambda$) is about 20 times higher than the IgG decay rate constant ($\mu$), the solution of eqn (2) can be simplified to

$$Ab(t) \approx \frac{p}{\lambda} AFC(t)$$  

The memory phase of the VSV-neutralizing immunoglobulin response is characterized by a stable IgG antibody titer ($Ab_{mem}$) and presumably by a stable frequency ($AFC_{mem}$) of AFCs. Setting $\frac{dAb}{dt} = 0$ in (2) then gives

$$Ab_{mem} = \frac{p}{\mu} AFC_{mem}$$

indicating that in the memory phase IgG titers ($Ab_{mem}$) depend on the number of memory AFCs ($AFC_{mem} = \frac{\mu}{p} Ab_{mem}$). For the time point when in the early phase of the VSV-neutralizing immunoglobulin response antibody levels are identical to the IgG titers observed in the memory phase ($Ab(t) = Ab_{mem}$), the right terms of eqns (3) and (4) can be set equal. Since the growth rate constant ($\lambda$) is about 20 times higher than the decay rate constant ($\mu$), the equation can be transformed to

$$\frac{(\lambda/p)Ab_{mem}}{\mu} \approx 20 \times \frac{(\mu/p)Ab_{mem}}{\mu}$$

indicating that in the early phase 20 times more AFCs are required to obtain the same IgG titers as observed in the memory phase.

Since the pioneering work of G. Bell (Bell, 1970, 1971), many mathematical models of B-cell activation and antibody production have been developed (Bruni et al., 1975; Bell et al., 1978; Merrill, 1978a, b; Mohler et al., 1980; Marchuk & Petrov, 1987; De Boer & Perelson, 1991; Bocharov & Romanyukha, 1994). The model of Bachmann and co-workers addressed numerical prerequisites for an anti-viral immunoglobulin response at an early and a late time point, however it did not attempt to describe the transition from the early to the late phase of the immune response. In the following section we present a model of the VSV-neutralizing immunoglobulin response which models such a transition smoothly and which also takes into account the influence of different infection doses of VSV, and the switch from IgM to IgG antibodies. The increase in the neutralizing activity of IgG antibodies over time was also considered.
2. The Mathematical Model

Our model consists of a system of nonlinear differential eqns (5)-(12) involving 13 different non-negative parameters (Table 1). It does not incorporate any immunological processes which, according to current knowledge, are not directly involved in the antibody formation, e.g. the T-cell response, macrophages, etc. For further simplification the following assumptions are made: (i) the humoral immune response is localized exclusively in the spleen; (ii) VSV does not replicate in peripheral tissues; (iii) after occupation of ≥30% of the viral glycoprotein determinants by neutralizing antibodies, free virus is eliminated; (iv) the pool of naïve VSV-specific B cells is maintained at a constant level by a balanced death rate and de novo generation rate; (v) VSV-specific precursor B cells are triggered directly by B-cell receptor cross-linkage via multivalent VSV binding; (vi) if available, T-cell help is not limited; (vii) the binding of VSV-specific antibodies to viral determinants is irreversible.

The initial values of the variables denoting the number of precursor B cells specific to VSV \((B_0)\) and the density of free virus \((V)\) can be freely chosen. A realistic value for the initial number of VSV-specific precursor B cells in an unprimed mouse \((B^*)\) is 80–300 cells (Bachmann et al., 1994). The initial density of free virus \((V)\) can be chosen as in a “real” biological experiment, e.g. low dose \((2 \times 10^4\) PFU), intermediate dose \((2 \times 10^6\) PFU) or high dose \((1 \times 10^8\) PFU) of VSV infection. The other variables in eqns (5)-(12), which denote the number of proliferating B cells \((B_i)\), the number of AFCs which produce either IgM \((A_{M_i})\) or IgG \((A_{G_i})\) and the concentrations of neutralizing IgM \((M)\) or IgG \((G)\) antibodies, respectively, are response variables taking initial value zero. The rates of change of each variable with time after i.v. injection of a certain dose of VSV to mice are:

\[
d B_0/\text{d}t = - \mu_{B_0}(B_0 - B^*) - \varepsilon V B_0 \quad (5)
\]

Activated B-cells

\[
d B_i/\text{d}t = \varepsilon V B_0 - q B_i \quad (6)
\]

Proliferating B-cells

\[
d B_i/\text{d}t = 2^{10/16} q B_{i-1} - q B_i \quad i = 2, 3, \ldots, 17 \quad (7)
\]

AFCs producing IgM

\[
d A_{M_i}/\text{d}t = q B_0 - \mu_{A_{M_i}} - s(t) A_{M_i} \quad (8)
\]

AFCs producing IgG

\[
d A_{G_i}/\text{d}t = s(t) A_{M_i} - \mu_{A_{G_i}} \quad (9)
\]

Neutralizing IgM antibodies

\[
d M_i/\text{d}t = f_1 A_{M_i} - \gamma_1 VM - \mu_M M \quad (10)
\]

Neutralizing IgG antibodies

\[
d G_i/\text{d}t = f_2 A_{G_i} - \gamma_2 VG - \mu_G G \quad (11)
\]

Virus clearance

\[
d V/\text{d}t = - \gamma_3 m^{-1} VM - \gamma_2 g^{-1} VG \quad (12)
\]

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In eqn (5) the homeostasis of VSV-specific precursor B cells in unprimed mice ($B_0 = B^*$) is due to a balanced death rate of unstimulated VSV-specific precursor B cells ($-\mu_B B_0$) and a constant de novo generation rate of virus-specific precursor B cells from the bone marrow ($\mu_B B^*$) described by the negative term $\mu_B(B_0 - B^*)$. Thus the cell death rate constant ($\mu_B$) is equivalent to a B-cell turnover rate of about 0.2 per day (Gray, 1988). According to the law of mass action the activation rate of VSV-specific B-cells ($\varepsilon V B_0$) is proportional to the density of the pathogen ($V$) and to the frequency of naive virus-specific B cells ($B_0$). The activation rate constant of naive B cells ($\varepsilon$) is inversely proportional to the time between virus binding and B-cell activation ($t_{on}$), which ranges between 1 and 3 hr, and to the concentration of VSV which is sufficient for the activation of a specific immune response ($V_{thr}$), which is in the order of $10^7$ virions, resulting in $\varepsilon = \frac{1}{(t_{on} \times V_{thr})}$ (Marchuk et al., 1991). The activation rate constant $\varepsilon$ has not been experimentally determined.

Once activated, B cells enter a phase of clonal expansion with rate ($qB_i$) lasting 2–3 days, during which they divide up to 10 times before differentiating into AFCs. This corresponds to an average of $2^{10} \approx 1000$ AFCs for each stimulated B-cell, and an average doubling time of 5–7 hr (Hodgkin et al., 1996). If doubling times were independent negative exponentially distributed random variables, it would be appropriate to model the expansion by 10 linear equations of the form $dB_i/dt = 2qB_{i-1} - qB_i$ with $q \approx 3$ per day. However, the actual distribution of cell doubling times can be expected to be more concentrated than the negative exponential distribution (Fig. 1); there is some indication of this to be deduced from (Hodgkin et al., 1996, Fig. 1). We use the 16 linear equations given in eqn (7) to represent the proliferation rate, with 2 replaced by $2^{10/16}$ and $q \approx 5$ per day. It should be noted that this is just a mathematical artifact which allows a complicated stochastic system to be satisfactorily approximated by a much simpler deterministic system; the number $2^{10/16}$ has no intuitive biological significance. The precise choice of 16 equations was made with reference to fitting Fig. 2(c).

In the remaining eqns (8) to (11), the death terms ($\mu_A A_M$), ($\mu_A A_c$), ($\mu_M M$) and ($\mu_G G$) are self explanatory, as well as the production rates ($f_A A_M$) and ($f_A A_c$) of IgM and IgG antibodies from AFCs. The half-life of AFCs is 2–3 days (Ho et al., 1986; Gray, 1988) resulting in an AFC death rate constant ($\mu_A$) of about 0.33 per day. The serum half-life of IgM is 28 hr (Fehr et al., 1997) resulting in an IgM decay rate constant ($\mu_M$) of about 0.8 per day, whereas the serum half-life of IgG is 5–7 days (Bachmann et al., 1994) resulting in an IgG decay rate constant ($\mu_G$) of about 0.125 per day. A plasma cell is estimated to produce $10^9$ to $10^{10}$ antibody molecules per day (Leanderson et al., 1992; Bachmann et al., 1994) resulting in a rate constant of IgM production ($f_A$) of about $2.5 \times 10^8$ IgMs per cell and day, and a five times higher rate constant of IgG production ($f_G$).

The rate at which IgM secreting AFCs switch to IgG production in presence of T-cell help is represented as $(\delta t) A_M$. The question of optimal IgM–IgG switch was explored in Perelson et al. (1980), on the basis that an IgM–IgG switch should minimize the total response time. An early IgM production is essential for survival at high antigen doses; the effectiveness of the
response is otherwise relatively insensitive to the timing of the switch (Perelson et al., 1980, p. 235 Fig. 6). We chose the immunoglobulin isotype switch function \( s(t) \) to fit the following two experimental observations: (i) IgG serum titers are not yet detectable on day 4 of the immune response to VSV but they are dominant \((\geq 3\) dilution steps) on day 8 compared with IgM serum titers (Bachmann et al., 1993); (ii) 4 days after i.v. infection with \(2 \times 10^6\) PFU of VSV, about 25% of the VSV-specific hybridomas secreted IgG antibodies, whereas on day 5 equal numbers of hybridomas secreting IgG- and IgM-antibodies were detected (Kalinke et al., 1996a). B cells which can be fused to obtain hybridomas have presumably recently been stimulated by the antigen, and therefore do not yet secrete significant amounts of antibody. Thus the distribution of IgG and IgM secreting hybridomas indicates the distribution of antibody subclasses found in the serum about 1 day later. We therefore modelled the isotype switch with the time variable switch function \( s(t) \) defined as follows: until day 3.5, \( s(t) \) is identical to zero, between day 3.5 and day 7 the switch function increases exponentially with rate 3 from about 1/200 up to 180, which then corresponds to an (effectively instantaneous) switching time of about 10 minutes. From day 7 on, \( s(t) \) remains constant.

\[
s(t): = \begin{cases} 
0 & \text{for } t \leq 3.5 \text{ days} \\
1/10 \cdot e^{(t-3.5)/3} & \text{for } 3.5 \leq t \leq 7 \text{ days} \\
180 & \text{for } t > 7 \text{ days}
\end{cases}
\]

If T-cell help is missing, e.g. in CD4\(^+\) depleted mice, the switch function \( s(t) \) is set to zero which results in an IgG antibody concentration of zero in eqn (11).

The rate at which IgM molecules bind to the virus \((\gamma_1 V M)\) is proportional to the density of free virus \((V)\) and to the serum concentration of neutralizing IgM antibodies \((M)\); the rate at which IgG molecules bind to the virus \((\gamma_2 V G)\) is modelled similarly. The rate constants of virus clearance by IgM antibodies \((\gamma_1)\) and by IgG antibodies \((\gamma_2)\), respectively, are directly proportional to the \textit{in vitro} neutralization rate constants \((K_{s1})\) of about \(10^{10}\) l\(^{-1}\) mol\(^{-1}\) (Zinkernagel, 1996; Kalinke et al., 1996a) and \((K_{s2})\) of about \(3 \times 10^8\) l\(^{-1}\) mol\(^{-1}\) (Kalinke et al., 1996a).

Equation (12) describes the rate of clearance of free virus \((V)\) from the serum by neutralizing antibodies according to the law of mass action. In normal mice, virus does not replicate outside neuronal tissues, so there is no need for a replication term. Free virus \((V)\) is neutralized by IgM antibodies \((M)\) with the rate \((\gamma_1 V M)\) which takes into account that the collision of one IgM antibody with one virion covers only a fraction \(m^{-1}\) of the VSV G-protein epitopes. The rate at which free virus \((V)\) is neutralized by IgG antibodies \((G)\) is described by the term \((\gamma_2 V G)\). To make a VS-virion uninfecious, at least 30% of its 1200 G-protein molecules have to be covered by neutralizing antibodies (Flamand et al., 1993; Kalinke et al., 1996b; Bachmann et al., 1997). This requires a minimum of \(~70\) pentameric IgM \((m^{-1} = 1/70)\) or \(~200\) monomeric IgG \((g^{-1} = 1/200)\) molecules being bound per virion.

3. Results and Discussion

The above model was used in computer experiments to simulate the kinetics of a primary VSV-neutralizing immunoglobulin response in the spleen of BALB/c mice after experimental injection of a low dose \((2 \times 10^6\) PFU), an intermediate dose \((2 \times 10^6\) PFU) and a high dose \((1 \times 10^6\) PFU) of VSV until about day 12 post-infection. Beyond day 12 the production of IgG antibodies from germinal center and bone marrow plasma cells is not taken into account. Figure 2 shows a typical computer simulation of eqns (5)–(12); closed circles (○) indicate data points taken from \textit{in vivo} experiments with BALB/c mice (Bachmann et al., 1993, 1994).

With the 16 subequations used in eqn (7) we could model the clonal B-cell expansion in a way that AFCs which secrete neutralizing IgM antibodies appear first 2 days after B-cell stimulation (Jacob et al., 1991), whereas the majority of terminally differentiated B cells appear as IgM secreting AFCs by day 4 post-immunization [Fig. 2(a)]. Between days 2 and 4 the frequency of AFCs increases by a factor of 10 from 15,000 to 20,000 to about 170,000 IgM producing cells, resulting in a
Fig. 2. Simulated VSV neutralizing immunoglobulin response of mice injected i.v. with 2 × 10⁶ PFU of live VSV-IND. (a) After a time lag of ~2 days post-infection, substantial amounts of AFCs begin to appear in splenic foci. From days 2 to 4 the frequency of IgM producing AFCs (solid line) increases by a factor of 10 to a maximum level of about 170,000 cells on day 4. Between days 4 and 6 nearly all AFCs switch in concert from IgM to IgG secretion due to the switch function s(t). Thus, IgG secreting AFCs (dashed line) peak around day 6 post-immunization. The model indicates that the number of VSV-specific AFCs declines from ~170,000 IgM producing cells on day 4 of the immune response via ~85,000 IgG producing cells on day 8 to below ~25,000 IgG producing cells from day 12 onwards. The experimentally determined number of AFCs per mouse in the spleen and bone marrow is about 100,000 cells (●) on day 8 (Bachmann et al., 1994); (b) substantial amounts of neutralizing IgM antibodies (≥10 µg ml⁻¹, lower dashed line) appear in the blood serum after day 2 post-immunization. Around day 4 the concentration has increased to about 50 µg ml⁻¹ (upper dashed line), and a maximum concentration of VSV-specific IgM molecules in the serum of about 80 µg ml⁻¹ is reached around day 5 post-infection from which on it declines exponentially at rate µM; (c) calculated neutralizing IgM antibody titer in −log₂ (titer × pre-dilution) representation fitted to data points (●) taken from experiments with BALB/c mice (Bachmann et al., 1993). A neutralizing activity of 600 µg⁻¹ for primary IgMs is assumed. The increasing flank of the graph calculated with the formula suggested in the Appendix fits the data points. From day 6 on IgM serum titers decline with about one dilution step per day because AFCs have switched to IgG production; (d) VSV-neutralizing IgG molecules appear from day 5 on only in presence of T-cell help. Concentrations of 10 µg ml⁻¹ (lower dashed line) are reached between days 5 and 6, concentrations of 50 µg ml⁻¹ between days 6 and 7, and a maximum serum concentration of about 90 µg ml⁻¹ is reached around day 10 post-immunization. The model suggests that only few IgG molecules are expended for virus clearance; (e) calculated neutralizing IgG antibody titer in −log₂ (titer × pre-dilution) representation fitted to data points (●) taken from experiments with BALB/c mice (Bachmann et al., 1993). The solid line indicates a calculation with an increasing neutralizing activity from 5 µg⁻¹ on day 5 to 600 µg⁻¹ on day 8; the dashed line shows the same calculation with a constant neutralizing activity of 5 µg⁻¹ between days 4 and 12.
frequency of AFCs in the spleen which is \( \sim 1000 \) times higher compared with the number of VSV-specific precursor B cells in unprimed mice \((B^* = 200)\). Due to the chosen switch function \( s(t) \) almost all AFCs switch in concert from IgM to IgG secretion between days 4 and 6. Thus, the number of AFCs declines from a maximum of \( \sim 170,000 \) IgM producing cells on day 4 via \( \sim 85,000 \) IgG producing cells on day 8 down to \( \sim 25,000 \) cells on day 12 after infection. The model indicates that, at the apex of the VSV-specific immunoglobulin response on day 4, about seven times more AFCs are active in antibody production as compared with day 12. The above results are in good accordance with the following experimental observations: VSV-neutralizing IgM serum titers can be detected from day 2 on, whereas VSV-neutralizing IgG titers appear between days 4 and 8 (Bachmann et al., 1993, 1994; Roost et al., 1995). At day 8 of the immune response about 100,000 VSV-specific AFCs were detected in the spleen of mice (Bachmann et al., 1994).

Protection of mice against primary VSV-infections depends on neutralizing antibody responses (Bachmann et al., 1993; Steinhoff et al., 1995; Zinkernagel 1996; Kalinke et al., 1996b). Because the growth rate of the IgM serum concentration is directly proportional to the number of IgM secreting AFCs during the first four days [eqn (10)], substantial amounts of VSV-neutralizing IgM antibodies \((> 10 \, \mu g \, ml^{-1} \, IgM)\) appear in the serum after day 2 post immunization. The maximum concentration of IgM molecules \((\sim 80 \, \mu g \, ml^{-1} \, IgM)\) is reached on day 5 post-infection [Fig. 2(b)] corresponding to about 10% of the IgM molecules in the serum then being specific to the virus, since the maximum serum concentration of IgM is \( \sim 900 \, \mu g \, ml^{-1} \) (Cogné et al., 1994). The IgM serum concentration then decays exponentially at rate close to \( \mu_m \) per day. In Fig. 2(c) calculated neutralizing IgM concentrations were transformed with formula (A.1) into neutralizing IgM serum titers and compared with the experimental observations (○). If we assume a neutralizing activity of 600 \( \mu g^{-1} \) for IgM molecules, we can see that between days 1 and 4 the calculated IgM serum titers rapidly increase by more than three dilution steps per day. The maximum of the IgM serum titer seems to be reached on day 5 post-immunization. From day 6 on, the model indicates a decline of neutralizing IgM serum titers of about one dilution step per day. This is because AFCs have switched to IgG production and no more IgM is secreted.

A computer simulation of the mathematical model in the case of T-helper cell depleted mice \([s(t)\) is set to zero] infected i.v. with \(2 \times 10^6\) PFU of live VSV-IND indicated that the IgM serum titers declined more slowly than in experiments in vivo. Figure 3(b) shows a clear misfit of \( \sim 4 \) dilution steps in the decline of the neutralizing IgM serum titer (solid line). This is because IgM is continuously produced by the AFCs during the exponential decline phase, and indicates that
the absence of T-helper cells seems to defer the decline of serum IgM by more than 2 weeks. If we assume a 10-fold reduced antibody secretion rate from day 6 on we get a perfect fit of the IgM antibody titer in T-helper cell depleted mice [Fig. 3(b), dashed line]. In strictly IL-6 dependent primary immunoglobulin responses a 10-fold reduction of the antibody secretion rate was observed in the absence of interleukin 6 (IL-6) (Muraguchi et al., 1988; Hilbert et al., 1989), which presumably is mainly produced by T-helper cells; however whether this explanation also holds true for the accelerated decline of VSV-neutralizing IgM titers in T-helper cell depleted mice is pure speculation and remains to be tested experimentally.

Protection against reinfestation depends on neutralizing IgG antibodies (Lefrancos, 1984; Zinkernagel et al., 1985; Bachmann et al., 1994; Kalinke et al., 1996a). VSV-neutralizing IgG molecules appear from day 4 on only in the presence of T-cell help. The model shows that protective levels of neutralizing IgG antibodies against VSV can be reached within the critical time of 5 to 6 days post-infection. Experiments indicate a minimal in vivo antibody concentration of 1–10 μg ml⁻¹ is necessary for protection by memory IgG (Bachmann et al., 1997). The model suggests that in vivo concentrations in the order of 100 μg ml⁻¹ IgG can be reached within 7 to 8 days if all virus-specific B cells are triggered [Fig. 2(d)]. An interesting detail of the mathematical solution is that IgG antibodies reach their protective concentration only after free virus has been eliminated from serum by IgMs (V ≤ 1). Thus, IgG does not seem to be directly involved in virus clearance. However, it is required for survival since the majority of CD4 depleted mice which do not develop IgG titers die within 8–12 days. Without IgG, some virus particles may survive at places inaccessible for IgM, even though virus is no longer detectable in serum. Thus, IgG may be required to prevent virus dissemination from such hiding-places into neuronal tissue. The maximum serum concentration of approximately 90 μg ml⁻¹ IgG is reached by day 10 post-infection and remains relatively stable between days 8 and 12.

In Fig. 2(e) neutralizing IgG serum titers are calculated and compared with the experimental observations (○). In contrast to IgM antibodies, we have experimentally determined values of neutralizing activities of primary (days 4 and 5), secondary (day 12) and hyperimmune (day 150) response IgG antibodies, indicating an increase in the neutralizing activity between primary IgGs (days 4 and 5) and secondary IgGs (day 12) (Kalinke et al., 1996a). These values and the calculated IgG concentrations were used in the formula (A.1) in the Appendix to transform serum antibody concentrations into corresponding neutralizing antibody titers. For a constantly low neutralizing activity of 5 μg⁻¹ over the whole investigation period, the calculated graph does not fit the data points [Fig. 2(e), dashed line]. If we assume an exponential increase in the neutralizing activity from 5 μg⁻¹ on day 5 to 600 μg⁻¹ on day 8 post-infection, the graph does fit the data points [Fig. 2(e), solid line]. The experimentally observed increase in the neutralizing activity between primary and secondary IgGs is taken into account in the denominator of formula (A.1) by a function h(t) similar to the isotype switch function s(t): the neutralizing activity of IgG antibodies is 5 μg⁻¹ until day 5; it increases exponentially from 5 to 600 μg⁻¹ between days 5 and 8 and then it remains at this level from day 8 on.

\[
h(t) = \begin{cases} 
5 & \text{for } t \leq 5 \text{ days} \\
1/5 \times e^{0.518} & \text{for } 5 < t \leq 8 \text{ days} \\
600 & \text{for } t > 8 \text{ days}
\end{cases}
\]

Thus, our model allows us to predict the time interval over which the neutralizing activity of primary IgGs should increase. Because the increase in the neutralizing activity could have followed other kinetics, other possible functions h(t) were investigated by computer experiments in Funk (1997).

As yet little is known about the mechanism that leads to the increase in the neutralizing activity of the IgGs. At the earliest from day 6 on, germinal center plasma cells begin to appear in the spleen (Bachmann et al., 1996) which may produce IgG molecules with higher neutralizing activities by using other germline gene fragments.
or by introducing somatic point mutations in the variable gene region (Kalinke et al., 1996a). Thus, it is possible that IgG molecules with low neutralizing activities from primary AFCs become continuously replenished by IgG molecules with higher neutralizing activities secreted by plasma cells. Although plasma cells may contribute to higher IgG serum titers, we did not take them into account in our model. A rough estimate of the number of plasma cells necessary to maintain a certain IgG antibody titer during the memory phase is given below.

The influence of the virus dose on the kinetics of B-cell activation and the frequency of AFCs was studied by running the model with various doses of VSV (Funk, 1997). Computer experiments indicated that for a low virus dose of $2 \times 10^4$ PFU, a weaker immune response is generated than for higher immunizing doses. For intermediate ($2 \times 10^6$ PFU) and high ($1 \times 10^8$ PFU) doses of VSV, IgM secreting AFCs peak on day 4 post-infection to higher maximum levels than IgG secreting AFCs on day 6. For low dose infections the populations of IgM and IgG secreting AFCs reach nearly the same maximum level because not all virus-specific precursor B cells are triggered by the antigen. This in turn leads to a lower maximum level in the population of IgM secreting AFCs. Furthermore, the numerical solution of the mathematical VSV-model shows that for antigen doses higher than $2 \times 10^6$ PFU, i.e. $10^7$ PFU, nearly the same immune response is generated as for $2 \times 10^6$ PFU because all VSV-specific precursor B cells are triggered. The larger number of IgM secreting AFCs compensates the shorter serum half-life of IgM compared with the serum half-life of IgG. This is in good accordance with experimentally observed data (Bachmann et al., 1994). One can speculate that evolutionary forces may drive selection for a value of VSV-specific precursor B cells that guarantees on average the survival of the infected host. It appears that the outcome of an infection with VSV is determined rather by the immunological status of the animal (e.g. $B^*$) than by the infective dose ($V$).

During the first 2 days post-infection the mathematical model indicates constant levels of VSV, but as soon as AFCs appear in the spleen a rapid decline in the concentration of free virus in the serum mediated by neutralizing IgM molecules can be observed (Fig. 4). Between days 2 and 4 more than 99% of the virus is neutralized by IgM resulting in an estimated serum half-life of VSV after the onset of a VSV-neutralizing antibody response of about 4 hr. Although, this result is in good accordance with experimental observations, new experiments indicate that free virus disappears in mice much faster than our model had suggested (A. Ochsenbein, pers. commun.). It is not clear whether this faster virus clearance is mediated by the interferon system, pre-existing natural antibodies or whether it depends on the “stickiness” of the virus which may lead to attachment of the virus to endothelial cells.

We estimated the number of plasma cells ($PC$) that is necessary to maintain a certain neutralizing IgG antibody titer during the rest of life. Since the IgG production rate by germinal center plasma cells ($f_{PC}$) is balanced by the decay rate of IgG molecules in the mouse serum ($\mu$), eqn (12) describing changes in the IgG concentration ($G$) could be modified as following:

Neutralizing IgGs

$$dG/dt = f_{PC} - \mu G = 0$$

![Fig. 4. Modelling virus clearance for low ($2 \times 10^4$ PFU), intermediate ($2 \times 10^6$ PFU) and high ($1 \times 10^8$ PFU) doses of live VSV-IND in BALB/c mice. The amount of VSV is given in plaque forming units (PFU). One PFU corresponds approximately to one single infective virus particle (Battigay et al., 1991). The model indicates a rapid decline in the concentration of free virus from day 2 on mediated by neutralizing IgM molecules, and free virus disappears from blood on day 5 post-infection ($V < 1$). The estimated half-life of free virus after the onset of the neutralizing IgM response is in the order of 4 hr. The model suggests identical kinetics of virus clearance in normal and T-helper cell depleted mice.](image-url)
Thus, for the number of plasma cells, one gets the rough estimation

\[ PC = \frac{\mu_e \cdot G}{f_s} \approx \frac{1/8 \cdot 2 \times 10^{14}}{10^6} \approx 25 \, 000 \]  

(14)

If we take into account that the neutralizing activity of IgGs increases over time (Kalinke et al., 1996a), one can see that the neutralizing IgG titer can slowly increase while the serum IgG antibody concentration can slowly decrease. Our estimated value for a constant IgG serum half-life of 1 week is \( \approx 25 \, 000 \) plasma cells, which can slowly decrease over time while the neutralizing activity increases. Experimentally determined frequencies of plasma cells are between 23,000 (day 21) and 16,000 (day 50) (Bachmann et al., 1994).

Given a constant number of plasma cells during the memory phase and a half-life of at least 7 days (Ho et al., 1986; Gray, 1988; Schittek & Rajewsky, 1990), approximately 3000 VSV-specific plasma cells enter IgG production every day to balance cell death. If we assume that a stimulated plasma cell divides eight times before it starts IgG secretion, every day between 5 and 20 VSV-specific B cells have to be triggered—probably by follicular dendritic cells presenting VSV—and proliferate to IgG secreting plasma cells to maintain constant serum titers for the rest of the life. This number is surprisingly small compared with 500 precursor B cells generated per second in the bone marrow of an adult mouse (Rolink & Melchers, 1991), 10% of which enter the pool of peripheral mature B cells resulting in a total of about \( 5 \times 10^4 \) newly generated mature B cells every day. If a memory B cell half-life of more than 7 days was assumed, as was suggested for long living bone marrow residing plasma cells with a half-life of \( \geq 90 \) days (Ho et al., 1986; Schittek & Rajewsky, 1990; Manz et al., 1997), the number of B cells which have to be triggered daily to maintain a lifelong antigen-dependent B-cell memory would be further significantly reduced.

### 4. Concluding Remarks

In this paper we present a mathematical model which describes the dynamics of a VSV-neutralizing immunoglobulin response in the spleen of mice. The model was formulated within the clonal selection paradigm and combines in vitro and in vivo data of the immunoglobulin response after experimental VSV-infection. It does fit the few data points determined experimentally by Bachmann and co-workers and allows prediction of values of any of the variables considered by the model in between measurement points. The model revealed that between days 4 and 5 after VSV infection about 10% of the total IgM molecules in the serum are virus-specific. The model predicts an increase in the neutralizing activity of primary response IgG antibodies between days 5 and 8. It suggests surprisingly low numbers of virus-specific B cells to be triggered daily to maintain protective immunoglobulin concentrations in the serum for life. Thus, the proposed mathematical model may serve as a starting point for further biological and mathematical investigation.

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### REFERENCES


MODELLING VSV-NEUTRALIZING ANTIBODY TITERS


APPENDIX

Calculation of Neutralizing Serum Antibody Titers

Serum antibody titers are derived from the numerically calculated IgM or IgG molecule concentrations in the following way: divide the starting dilution concentration, usually 1 \( \mu \text{g ml}^{-1} \), by the (estimated) neutralizing activity in \( \mu \text{g}^{-1} \) and by the (calculated) antibody concentration in \( \mu \text{g ml}^{-1} \). If serum is pre-diluted, the calculated antibody concentration has to be divided by the pre-dilution factor. Taking the negative logarithm to the base of 2 of this value, one gets the \(-\log_2 (\text{titer} \times \text{pre-dilution})\) representation. Thus, the transformation formula for a pre-diluted serum is

\[
-\log_2 (\text{titer} \times \text{pre-dilution}) = -\log_2 \left( \frac{\text{starting concentration}}{\text{neutral.activity} \times \text{antibody concentration}} \right). \quad (A.1)
\]