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Ratio-dominance model of suppression: an analysis by limiting dilution

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Summary. A theoretical framework is presented which explores two models of suppressor cell–target cell interactions in T-dependent antibody responses. The first is the full-dominance model, in which a single or limited number of suppressor cells can entirely suppress an immune response irrespective of the multiplicity of other effector cells present. The second is the ratio-dominance model, in which a suppressor cell is capable of inactivating only a certain number of target cells. Thus, the multiplicity of target cells in a given microculture well influences the degree of suppression. Both models are evaluated using limiting dilution analysis and two systems are explored. In the first model, suppressor cells alone are titrated into microculture wells containing all other cells required for an immune response. In the second, suppressor cells are added from populations containing a mixture of helper T cells as well as suppressor cells. This latter type of analysis is similar to that in which populations of T cells primed to alloantigens in mixed lymphocyte cultures are analysed for positive (help) and negative (suppression) allogeneic effects. The analysis allows us to conclude that such suppressor cells operate via a mechanism best described by a ratio-dominance model.

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INTRODUCTION

It has become increasingly clear over the past several years that the immune system is composed of variety of functional subpopulations of lymphocytes and that the immune response to any antigen is regulated by various cell types of differing specificities (Cantor & Boyse, 1977; Eichmann, 1978; Gershon, 1974; Herzenberg, Okumura, Cantor, Sato, Shen, Boyse & Herzenberg, 1976). Despite the growing appreciation of the role of suppressor T cells in the regulation of immune responses, most of the work concerning suppressor cells remains descriptive and relatively little information regarding their cellular targets or their mode of action is available. We have developed a system in which the various activities of helper T cells, B cells and suppressor cells can be investigated (Corley, Kindred & Lefkovits, 1978; Aarden, Corley, Söderberg & Lefkovits, 1980). In the preceding paper (Aarden *et al.*, 1980), we analysed by limiting dilution the suppression of B-cell responses mediated by mixed lymphocyte reaction—primed cells (MLR-primed cells). The observations which are pertinent to the present discussion are as follows:

(1) When MLR-primed cells are added to B cells in the presence of antigen, a strong positive allogeneic effect is observed at relatively low inputs of primed

cells resulting in specific antibody formation. At higher cell input, this positive allogeneic effect is reversed and suppression takes place.

(2) Limiting dilution analysis revealed that the suppressive activity was mediated by a cell with a restricted frequency, i.e. at certain cell inputs beyond those which give rise to maximum numbers of responding cultures, suppressive effects fluctuate and some wells remain positive while others, receiving the same number of cells, are rendered negative. Furthermore, these latter cultures can respond to a second antigen, as we showed that the responses to sheep and horse erythrocytes were suppressed independently.

(3) The target of suppression is a B cell rather than a helper T cell. Moreover, the degree of suppression is inversely proportional to the B-cell input. The higher this input, the more difficult it is to achieve suppression.

In this paper, we present a theoretical framework for investigating the stoichiometric relationships between suppressor cells and their targets. This discussion is modelled around the results we have observed in dissecting positive and negative allogeneic effects. However, the results can be used as a general guide in the analysis of suppressor cell–target cell interactions in other systems, thereby providing a degree of versatility and control not previously possible in the study of suppressor cells and their mode of action.

MATERIALS AND METHODS

Limiting dilution analysis

Limiting dilution analysis is based on the measurement of the frequency of rare events. The fact that we find a fluctuation of the antibody response in the suppressive part of the dose–response curve of MLR-primed cells added to B cells enables us to apply this approach. In order to use limiting dilution analysis, however, one must ensure that the observed fluctuations are due to the Poissonian inhomogeneity* of the titrated population and not of the detection system. Thus, for analysing cells which suppress T-dependent immune responses, at least three cell types must be in excess in culture: helper T cells, B cells, and accessory cells. We have fulfilled these criteria by using splenocytes from nude mice as a saturating source of B cells (at least five antigen-specific precursors per well) and accessory cells; helper T cell activity is provided in excess in the MLR-primed population (Corley *et al.*, 1978; Aarden *et al.*, 1980) or by adding optimal amounts of T-cell replacing factors (Aarden *et al.*, 1980). Our models are therefore based on the assumption that the detection system is homogeneous and that we are measuring the inhomogeneity of suppressor cells in the titrated MLR-primed population.

*Poissonian inhomogeneity refers to the variation of the actual numbers of cells per well. With increasing numbers of cells, the mean of the specific cells per microculture increases and the inhomogeneity of the system decreases. The expression "inhomogeneity" is not to be confused with "cellular heterogeneity".

RESULTS

Poisson distribution

If precursor cells are randomly distributed in the titrated population, the Poisson distribution can be applied, and, from the zero term of the Poisson distribution, the frequency of these cells can be calculated. The application of Poisson distribution to suppressor cell titrations differs from the analysis of titrated T helper (Th) cells or B cells. Therefore, it is informative to review the analysis of these latter cell types prior to discussing suppressor cell titrations.

Titration of helper T cells or B cells in T-dependent antibody responses

T-dependent antibody responses require the interaction of at least two different classes of lymphocytes: Th cells and antigen-specific precursor B cells. Let us consider a situation in which each microculture contains all cell types needed for response, including excess B cells and accessory cells, except the one which is the subject of assay. Let this cell be a Th-cell precursor which is added with such a low multiplicity that a considerable fraction of microcultures will not contain such a cell. If these precursor cells are randomly and independently distributed throughout the microcultures, the number of Th precursor cells per well will follow a Poisson distribution. The mean number of precursor cells can be calculated from the observed proportion of negative cultures using the Poisson formula,

$$F_r = \frac{u^r}{r!} \cdot e^{-u} \qquad (1)$$

where F_r is the fraction of cultures containing r Th precursor cells, and u is the mean number of Th precursor cells per well (u_{Th}). The zero term of the Poisson distribution is

$$F_0 = e^{-u} \quad (2a)$$

and the logarithm of the equation is

$$u = -\ln F_0 \quad (2b)$$

which means that the negative logarithm of the fraction of non-responding cultures (i.e. those containing no Th precursor cell) is *linearly proportional* to the input of Th precursor cells per well. Note that the only 'defined' cultures are those that do not respond, because they do not contain any Th cells, a responding culture may contain 1, 2 or more Th cell precursors.

If the negative logarithm of the fraction of non-responding cultures ($-\ln F_0$) is plotted on the y-axis, and the cell input is plotted on a linear scale on the x-axis, the experimental points are expected to fit a straight line (Fig. 1). A similar type of analysis can be applied to B-cell titrations, as long as Th-cell precursors and accessory cells are not limiting.

Titration of suppressor cells

For titration of suppressor T (Ts) cells, the situation will change in two respects. First, the population of cells needed to be present in excess has to contain not only B cells, accessory cells and antigen, but Th precursor cells have to be supplied as well. Second, the zero term of the Poisson distribution is defined differently from that used to identify Th or B cells. The zero term of the Poisson distribution reflects those cultures that do not contain a suppressor cell, i.e. the cultures that respond and is defined as

$$F_+ = e^{-u} \quad (3a)$$

and u is the mean number of Ts precursor cells per well (u_{Ts}). Thus, for titration of suppressor cells, the F_+ rather than F_0 must be plotted (Fig. 2).

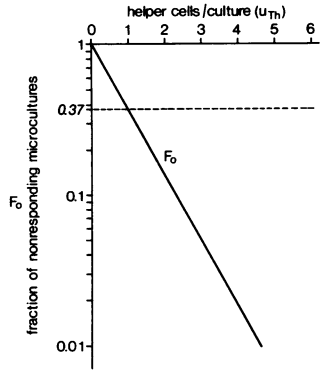

Figure 1. Titration of helper T cells into microcultures containing all cells required for the response except the helper cell which is being titrated. This cell is independently and randomly distributed in the titrated population, and the presence of one helper cell is sufficient to convert a non-responding culture to a responding one (single-hit kinetics). Note that for titrations of helper cells, the defined cultures are those that do not respond (F_0), since responding cultures can contain one or more helper T cells.

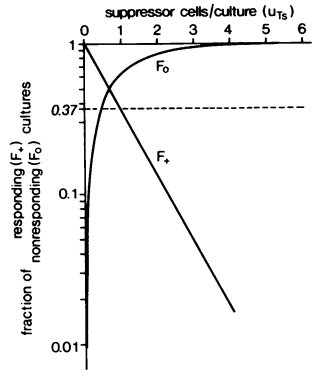

Figure 2. Titration of suppressor cells into microcultures containing all other cell types required for a response. The F_0 curve represents the increase in the number of non-responding cultures with increasing numbers of titrated cells added. The F_+ curve represents an example of single-hit kinetics for the suppressor cell titration. Note that the defined cultures for analysis of suppressor cells are the responding (F_+) cultures, in contrast to defined cultures for titration of helper cells.

'Hittedness' of the suppression: titration of suppressor cells

In this section, we shall examine a situation in which the cell population to be analysed contains only suppressor cells but no other immunologically active cells relevant to the system being measured. Th cells, B cells and accessory cells are provided in excess from an external source. In Fig. 2, the straight line shown when the fraction of responding cultures is plotted describes an experimental situation which is termed a *single-hit event*. The prerequisite for obtaining experimental results which are best described by single-hit kinetics is that a *single* suppressor cell, if

present in a microculture, will inactivate the culture irrespective of the multiplicity of specific Th cells and B cells in the well.

If the above assumption is not fulfilled, the experimental curve will deviate from the straight line. For example, if two suppressor cells or more are needed for suppression, cultures containing no suppressor cells and cultures containing a single suppressor cell will respond. In this case, the fraction of responding cultures will be described by the sum of the zero term and first term of Poisson distribution

$$F_+ = (1 + u_{Ts})\, e^{-u_{Ts}} \qquad (3b)$$

If suppression is achieved only in cultures which contain three or more suppressor cells, the fraction of responding cultures will be described by the sum of the zero, first and second term of the Poisson distribution:

$$F_+ = (1 + u_{Ts} + \frac{u_{Ts}^2}{2})\, e^{-u_{Ts}} \qquad (3c)$$

The corresponding curves are shown in Fig. 3a and b. In all of these situations the presence of a certain number of suppressor cells per well will render a culture negative, irrespective of the further contents of the well. These results reflect what we term the *Dominance Model* of suppression.

The dominance model of suppression can be distinguished from a second type of suppressor model in which the multiplicity of other effector cells in the microculture well does influence the degree of suppression. In our studies of suppressor cells in MLR-primed populations, the multiplicity of B cells, which are the cellular targets of suppression, has a striking effect on the degree of suppression (Aarden *et al.*, 1980). This indicates that a suppressor cell is capable of inactivating only a limited number of B cells. When a high multiplicity of B cells is used, a single suppressor cell will be unable to prevent a culture from responding. The dominance of suppression, therefore, will be dependent on the ratio of B cells to Ts cells, and we therefore call this a *Ratio-Dominance Model.*

For the calculation of the zero term of the Poisson distribution we have to introduce the variable a, which is the number of suppressor cells required to inactivate a culture,

$$a = \frac{u_B}{n_B} \qquad (4a)$$

n being the number of B cells inactivated by a single

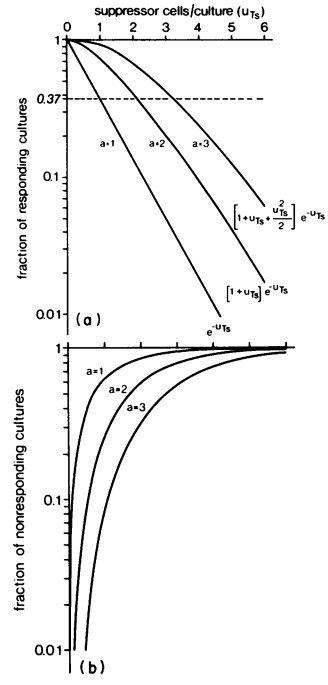

Figure 3. Titration of suppressor cells into microcultures containing all other cell types required for a response. A certain number of suppressor cells are necessary to prevent a culture from responding. For $a=1$, a single suppressor cell will inactivate the culture; $a=2$, two suppressor cells are required; $a=3$, three suppressor cells are required. The formulae represent the relevant terms of the Poisson distribution which describe the three curves. (a) The number of titrated suppressor cells are plotted as a function of the fraction of responding cultures; (b) plotted as a function of non-responding cultures.

suppressor cell. The sum of the fractions of cultures which will have less than a cells is

$$\left(1 + u_{Ts} + \frac{u_{Ts}^2}{2} + \cdots + \frac{u_{Ts}^{a-1}}{(a-1)!}\right) e^{-u_{Ts}} \qquad (5)$$

In the case of suppressor cells acting on Th cells rather than on B cells the argument is similar, and the number of suppressor cells required to inactivate a culture will be again a

$$a = \frac{u_{Th}}{n_{Th}} \qquad (4b)$$

n being the number of Th cells inactivated by a single suppressor cell.

Thus, in both instances the fraction of responding cultures will be

$$F_+ = \left(1 + u_{Ts} + \frac{u_{Ts}^2}{2} + \cdots + \frac{u_{Ts}^{a-1}}{(a-1)!}\right) e^{-u_{Ts}} \quad (5)$$

For example, if the multiplicity of B cells per culture is 10, and the number of B cells inactivated by a single suppressor cell is 5, then the number of suppressor cells required to inactivate a culture is $a = \frac{10}{5} = 2$. In this example, the definition of F_+ is:

$$F_+ = (1 + u_{Ts})\, e^{-u_{Ts}}$$

which is a two-hit curve.* Note that when all effector cells are in excess in a microculture well, and only suppressor cells are titrated, the shape (hittedness) of the curve alone will not distinguish between a dominance or ratio-dominance mechanism of suppression. This requires that the effect of the multiplicity of target cells on the degree of suppression be known. This added requirement can change when both Th cells and Ts cells are supplied only in the titrated population.

'Hittedness' of suppression: mixture of T helper and T suppressor cells

In the previous section we have dealt with a type of problem in which the analysed cell population contains suppressor cells, but no other immunologically active cells. Now we examine cases where the analyzed cell population contains a mixture of Ts cells and Th cells as well as other (inert) cells. The interesting feature of such titration curves is that at a low input of the titrated cells the 'helper portion' is revealed (as long as their frequency is higher than that of Ts cells) while at higher input the suppressor activity prevails.

Let us first analyse the system with respect to the *dominance model* of suppression. The following assumptions will be made: (a) B precursor cells and accessory cells are supplied in saturating doses from an exogenous source; (b) Th cells are introduced only in the titrated samples; (c) a single suppressor cell, if present in the culture, will inactivate the whole culture.

*It should be noted that the Poissonian component of the fluctuation of u_B was disregarded from the calculation, but might have to be taken into account in a practical analysis. However, as long as the multiplicity of target cells is sufficiently large compared to the value a, the contribution of the target cell fluctuation on the results would be negligible.

The fraction of wells not containing suppressor cells will be $e^{-u_{Ts}}$, and similarly the fraction of wells not containing helper cells is expressed as $e^{-u_{Th}}$. The fraction of wells not containing suppressor cells, but containing at least one helper cell is the product of the two respective probabilities

$$(1 - e^{-u_{Th}})\, e^{-u_{Ts}},$$

and the fraction of nonresponding cultures will be:

$$F_0 = 1 - (1 - e^{-u_{Th}})\, e^{-u_{Ts}} \quad (6a)$$

$$F_0 = 1 - e^{-u_{Ts}} + e^{-(u_{Th} + u_{Ts})} \quad (6b)$$

Th and Ts are introduced in the same sample, thus the average ratios ($R = u_{Th}/u_{Ts}$) of the two kinds of cells is constant throughout the whole titration. Thus,

$$F_0 = 1 - e^{-u_{Ts}} + e^{-(R+1)u_{Ts}} \quad (6c)$$

or

$$F_0 = 1 - e^{-\frac{1}{R}u_{Th}} + e^{-\frac{R+1}{R}u_{Th}} \quad (6d)$$

In Figs 4–6, curves of the combined helper and suppressor cell titration are shown with three different ratios of Th/Ts cells. Both F_0 and F_+ curves are drawn. In each case, the frequency of Th cells is equal to or greater than the frequency of Ts cells. In

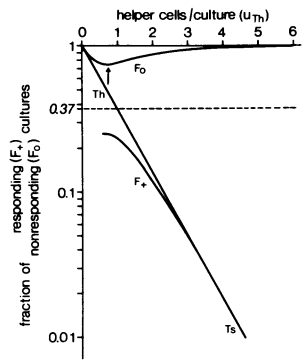

Figure 4. Titration of a population of lymphocytes containing both helper and suppressor cells; culture wells contain only B cells and accessory cells as well as antigen. Presence of a single helper cell in the microculture will activate the culture provided no suppressor cell is present. Presence of a single suppressor cell in the microculture will inactivate the culture irrespective of the presence of helper cell. The ratio of helper/suppressor cells in the titrated population is 1. The arrow points to the 'minima' calculated from the zero term of the first derivation of Equation 6. See text for details.

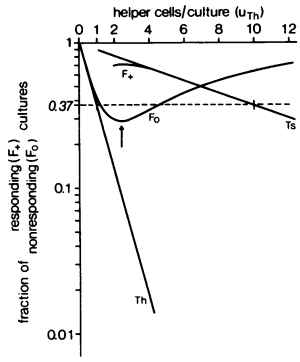

Figure 5. Same as Fig. 4, except that the ratio of helper/suppressor cells in the titrated populations is 10.

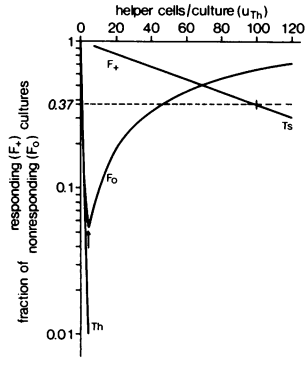

Figure 6. Same as Fig. 4, except that the ratio of helper/suppressor cells in the titrated population is 100.

the dominance model, the quotient of the slopes of the two curves (F_0 and F_+) is the chosen ratio of u_{Th}/u_{Ts}. Furthermore, for each R, there is a defined 'minimum' which can be calculated from the zero term of the first derivation of equation 6:

$$\frac{dF_0}{du_{Th}} = \frac{1}{R} e^{-\frac{1}{R}u_{Th}} - \frac{R+1}{R} e^{-\frac{R+1}{R}u_{Th}} \quad (7)$$

$$\frac{dF_0}{du_{Th}} = 0 \text{ (for minima)}$$

It follows that

$$\frac{1}{R} e^{-\frac{1}{R}u_{Th}} = \frac{R+1}{R} e^{-\frac{R+1}{R}u_{Th}}$$

$$e^{u_{Th}} = R + 1$$

$$u_{Th} = \ln(R + 1)$$

Thus, for the titration curves shown in Figs 4–6 the minima are:

for $R = 1$ $u_{Th} = \ln 2 = 0{\cdot}69$ $F_0 = 0{\cdot}75$ (Fig. 4)
for $R = 10$ $u_{Th} = \ln 11 = 2{\cdot}40$ $F_0 = 0{\cdot}29$ (Fig. 5)
for $R = 100$ $u_{Th} = \ln 101 = 4{\cdot}62$ $F_0 = 0{\cdot}054$ (Fig. 6)

which are indicated by arrows on the corresponding figures. Thus, even at a Th/Ts ratio of 100:1, the minima should never fall below an F_0 of 0·054. This clearly is in contrast to the results we have obtained using MLR-primed populations as a source of both Th and Ts cells. In these experiments, the minima in the Th portion of the curve often approach zero.

To calculate theoretical curves based on the ratio-dominance model, the following assumptions are made. (a) B precursor cells are supplied with a known multiplicity, such that the criteria for a saturating dose are fulfilled; accessory cells are abundantly present. Both cell types are provided from an exogenous source. (b) Th cells are introduced only in the titrated samples. (c) One suppressor cell is capable of preventing the response of only a certain number of B precursor cells.

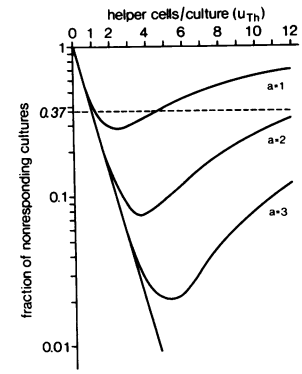

Figure 7. Titration of a population containing helper cells and suppressor cells in a ratio 10 ($R=10$), the curves predict the outcome if the suppressor cell–target titration follows a multi-hit mechanism. A single helper cell is capable of activating a culture, while one, two, or three, suppressor cells are required for inactivation. These theoretical curves resemble the experimental curves presented in Aarden *et al.* (1980), where the multiplicity of target cells (B cells) decisively influenced the titration pattern. This interdependence of the titrated cells and target cells is termed ratio-dominance.

The fraction of wells not containing suppressor cells will be $e^{-u_{Ts}}$, and the fraction of wells not containing helper cells $e^{-u_{Th}}$, as defined earlier. The number of suppressor cells required to inactivate a culture is chosen as a (Equation 4). The fraction of cultures which will have less than a cells is

$$\left(1 + u_{Ts} + \frac{u_{Ts}^2}{2} + \cdots + \frac{u_{Ts}^{a-1}}{(a-1)!}\right) e^{-u_{Ts}}$$

while the fraction of wells containing less than a suppressor cells but containing at least one helper cell is

$$F_+ = \left(1 + u_{Ts} + \frac{u_{Ts}^2}{2} + \cdots + \frac{u_{Ts}^{a-1}}{(a-1)!}\right)(1 - e^{-u_{Th}})\, e^{-u_{Ts}}$$

Figure 7 shows three theoretical titration curves based on the ratio-dominance model. Note that in the ratio-dominance model the position of the minima depends on the multiplicity of target cells (u_B or u_{Th}) which can be inactivated by a certain number of suppressor cells (n_B or u_{Th}).

DISCUSSION

Critical to our understanding of the mode of action of suppressor cells are experiments which are informative in two respects. First, the cellular or soluble target for the suppressor cell must be unequivocally identified. Second, the stoichiometric relationship between suppressor cells and their targets needs to be understood. In the previous paper, we designed a series of experiments which allowed us to identify the target cell of suppression mediated by MLR-primed lymphocytes (Aarden *et al.*, 1980). In the current paper, a theoretical framework was developed in which suppressor cell–target cell interactions can be analysed. With respect to MLR-primed suppressor cells, we have concluded that their target cell is a B cell (Aarden *et al.*, 1980). Moreover, the analysis presented in this paper allows us to conclude that a single suppressor cell can inactivate only a limited number of B cells. Thus, the degree of suppression observed is dependent only on the multiplicity of B cells in a well and on the maximum number of B cells capable of being suppressed by a single Ts cell. These results fit what we have termed the ratio-dominance model of suppression.

While the basis for developing the theoretical models presented in this paper stem from our work using MLR-primed populations, they have been generalized such that they can be used as a framework for analysing suppressor cell–target cell interactions in other systems. It remains to be determined if all suppressor cells operate in a ratio-dominance manner, or if a suppressor cell which exerts a truly dominant form of suppression exists. It is of interest to speculate that the form of suppression might vary depending on the suppressor cell's target, such that suppression of B cells and Th cells might exhibit different properties.

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