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Computational models in immunological methods: an historical review

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Abstract

The utilization of computational models in immunology dates from the birth of the science. From the description of antibody–antigen binding to the structural models of receptors, models are utilized to bring fundamental understandings of the processes together with laboratory measurements to uncover implications of these data. In this review, an historical view of the role of computational models in the immunology laboratory is presented, and short mathematical descriptions are given of fundamental assays. In addition, the range of current uses of models is explored — especially as seen through papers which have appeared in the *Journal of Immunological Methods* from volume 1 (1971/1972) to volume 208 (1997). Each paper which introduced a new mathematical, statistical, or computer simulation model, or introduced an enhancement to an instrument through a model in those volumes is cited and the type of computational model noted. © 1998 Elsevier Science B.V. All rights reserved.

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1. Introduction

Immunology as a science is usually dated from Metchnikoff's paper on the relationship between phagocytic cells and anthrax in 1884 (see Silverstein, 1989; Metchnikoff, 1893). In response to that proposed cellular role in immunity, studies on the properties of blood serum (the humoral alternative) by Nuttall in 1888 demonstrated that a substance in the blood, named 'alexin' by Buchner (later 'complement' by Ehrlich) had the ability to kill some microorganisms. As the humoral branch of the immune response was elucidated, identification of specific antibodies and their properties quickly followed. The precipitin reaction by Kraus in 1897, bacterial agglutination by Gruber and Durham in 1896, and the titration of anti-diphtheria antibodies and diphtheria toxin by Ehrlich also in 1897, began the science of immunochemistry (Arrhenius, 1907) and also initiated a quantitative aspect to immunology — moving the science for the first time beyond the description of phenomena. That is, immunology began to move from a collection of somewhat unrelated observations in vivo and in vitro, to in vitro demonstrations of these phenomena using

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well-defined laboratory procedures and assays. The standardization and reproducibility of the phenomena through these procedures provided the proper stage for experiments and theoretical discussions as to mechanism. It is easier to explain what happens in a reproducible controlled in vitro assay than to attempt to understand the nature of a similar phenomena in an intact animal. Computational models in immunology first arose from these humoral roots in the descriptions of complement fixation and antibody–antigen interactions (Bell and Perelson, 1978).

The phrase 'computational model' does not have a simple definition. 'Model' of necessity implies a simplification of the system under study, while 'computational' entails the use of some form of quantitative technique (sometimes several of them) to deduce information relating to the system. For instance, in a particular setting mathematical and statistical ideas could be used to create a computer simulation. However, a computational model is more than a presentation of data gathered through graphs and tables, and more than descriptive statistical quantities (e.g. mean and S.D.) computed from the data. The use of a computational model involves an attempt to use both data gathered, and assumptions and knowledge of the system under study to infer additional information regarding the system. For instance, a computer simulation could be used to show that the data gathered is consistent or inconsistent with a particular hypothesis. The implications arising from the data and assumptions are 'predictions' made by the model. If the assumptions are true and the model was carefully constructed, the predictions should be eventually observed. These predictions are a natural way in which new experiments are suggested as part of the scientific method. Note that verifying the predictions does not prove that the assumptions are true, only that they may not be inconsistent with observations.

In the study of a complex system, such as the immune system, models are essential in the testing of competing hypotheses. Due to the complexity of such a system, implications of a collection of hypotheses (or even their internal consistency) cannot be readily determined. Any model, however, is a simplification of the system under study, and that simplification, along with the logic of mathematics and statistics, allows the exploration of this simplified system to discover the nature of the implications of the hypotheses and assumptions on which the model is based. Furthermore, being able to construct a computational model based on a collection of hypotheses establishes the consistency and completeness of the hypotheses, meaning that there are no internal contradictions, and that these assumptions are at least sufficient to specify a simplified version of the system.

The simplifications involved in the modeling process make the applicability of a particular model to a system often a matter of contention. A system does not have a single model that 'describes' it, much as the fact that no two painters will paint the same scene in the same way. To evaluate a model critically, the assumptions and the purpose of the model need to be examined, as well as the reasonableness of the results obtained from applying the model.

A computational model describes the relationships between elements of a system which can be quantified or coded for in some way. These elements are most often numerical (as with antibody concentrations), but also could be lines connecting dots (a graph) as seen in descriptions of the precipitin reaction, strings of letters as in libraries of DNA fragments or primary structures of proteins, or images constructed through an imaging system. The elements of a system as they appear in a model of that system are simplified, less complicated representations or abstraction of the true objects, much as a cartoon figure represents a human. A computational model consists of these simplified elements and the specification of the relationships between the elements. These relationships may be based on theoretical considerations or empirical findings. These relationships are described through equations of various types, transition rules which describe which transitions are allowable and the probabilities of these transitions, relationships in a database, and other ways. The decision as to which elements of the system need to be described in a model, and the mathematical nature of the relationships are de-

termined by the training and experience of the modelers, and their knowledge of the system.

There has been an increasing use of computer software, both on a desktop and imbedded within instruments, for data analysis, storage, and display. Often the use of computational models on which the software is based (and the assumptions and simplifications that use implies) is not apparent to the user of these systems. It is hoped that through these examples and references, an increased awareness of the role and implications of the use of computational models in immunological methods will result.

After two short historical examples, brief discussions of the main areas for the use of computational models as seen through papers that have appeared in the Journal from volume 1 number 1 (1971) to volume 208 number 2 (1997) are given. Although an attempt was made to find all papers which fit the criteria of the use of a (new) computational model as an integral part of the paper, there are certain to be some omissions. Approximately one in 50 papers in the Journal over that period of over 7100 published fit the criteria used. These papers were grouped, sometimes with difficulty, into categories to further indicate the wide range of computational tools employed and to indicate how certain approaches tended to be used for particular methods. The author wishes to apologize to authors both for omissions of papers of note, and of commission for the immunological and computational classifications given. The complete text of this paper is available from the Journal web site, Computational Models in the Immunology section. Each of the references from the Journal has a hypertext link to its abstract.

2. Historical examples

2.1. Complement fixation

Leschly (1914) demonstrated the empirical relationship that the fraction (y) of the total of coated red cell lysed, hemolysis, as a function of complement (serum) added (x) followed a sigmoidal (S-shaped) curve as in Fig. 1. Transformations of the data which generate such a curve often suggest functional forms for these curves — usually by determining which transformations produce linearity in plots of the transformed data. In this case, von Krough (1916) showed that the logarithm of the amount of complement added [$\log(x)$] is plotted vs. the logarithm of the fraction of cells lysed over the fraction non-lysed

$$[\log(y/1-y)]$$

yields a straight line in those two transformed variables, giving the slope and intercept the names $1/n$ and $\log(K)$, respectively (names being suggested through a proposed mechanism),

$$\log(x) = \log(K) + \left(\frac{1}{n}\right)\log\left(\frac{y}{1-y}\right). \quad (1)$$

Using the properties of the logarithm, this relationship can also be written as

$$x = K\left(\frac{y}{1-y}\right)^{1/n} \quad (2)$$

The slope and the intercept in Eq. (1) are parameters determined by the line giving the best least squares fit (linear regression) through the transformed data in Fig. 2.

The presence of the parameters in the functional form for the relationship in Eq. (2) is important for two reasons: First, the values of the parameters determined by the fit allow a formal characterization of a data set. In comparing two sets of data, one may have a 'larger K', for

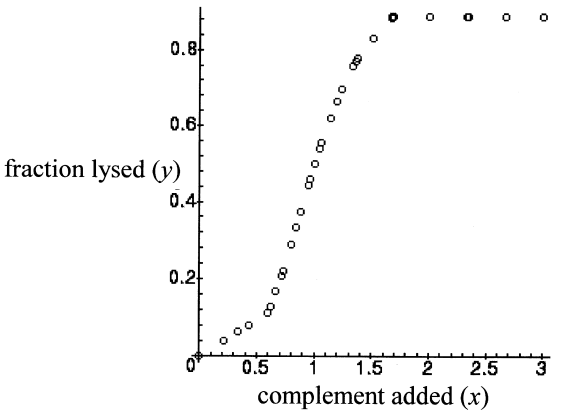

Fig. 1. Typical sigmoidal curve.

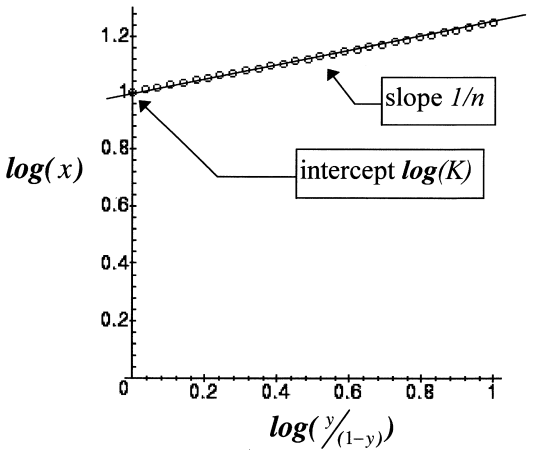

Fig. 2. Linearity of the sigmoidal data in Fig. 1 after the von Krough transformation.

instance. Secondly, the functional form may also suggest a mechanism for the lytic event. In the case of complemented-mediated lysis, fitting the sigmoidal curve lead eventually to a one-hit theory of hemolysis (Mayer, 1961; Rapp and Borsos, 1970). A modern use can be seen in Bengali et al. (1980).

Dealing with sigmoidal curves is a standard exercise in many assay systems. Most methods attempt to discover a transformation which will result in (near) linearity as above. Two of the most used are probit and logit. There are comprehensive monographs dedicated to each, Finney (1971) for probit analysis, and Ashton (1972), for logit, for example. These and other transformations are discussed in Govindarajulu (1988). A generalization of this approach are general linear models (GLM), which are extensions of the idea of linear regression (Liao, 1994). A recent review of estimating the relative potency of vaccines by Siev (1997) represents an example of using GLM in this setting.

The logit transformation assumes a logistic form for the sigmoidal curve,

$$y = \frac{1}{1 + e^{-(\alpha + \beta x)}}, \tag{3}$$

for some choice of the parameters α and β. The logit transformation (Berkson, 1944) is

$$\text{logit}(y) = \log_e \frac{y}{1-y},$$

which is a linear function of x,

$$\text{logit}(y) = \log_e \frac{y}{1-y} = \alpha + \beta x.$$

when the data has this logistic form. A plot of a typical logistic sigmoidal function and logit(y) are given in Fig. 3a,b.

The probit transformation (Bliss, 1934) uses the (cumulative) distribution function (CDF) of the normal distribution with a mean of 5 and S.D. = 1 to transform sigmoidal data which may be the CDF of a normal distribution with unknown mean μ and S.D. σ. The relationship between the CDF used by probit and that of the familiar 'z' scores (which have a mean of 0) is that five is added to z to get a probit, insuring that the probit is positive. As no closed functional form for the normal

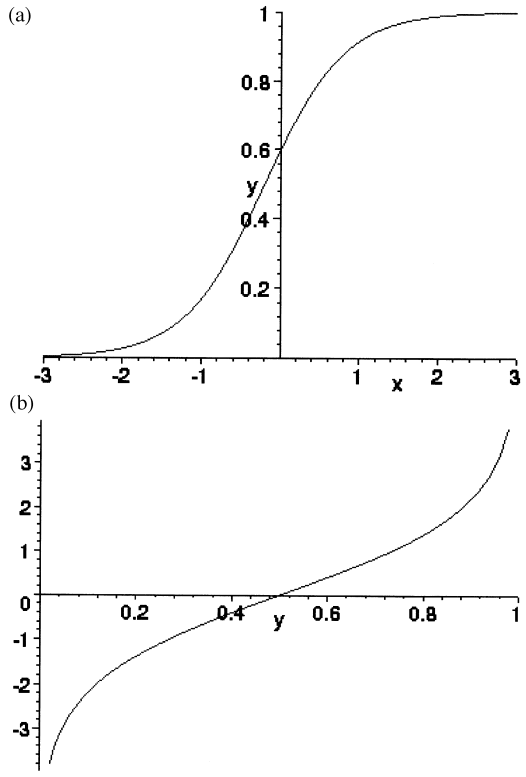

Fig. 3. (a) Typical logistic sigmoidal function. Here $\alpha = 0.4$ and $\beta = 2$. (b) A graph of logit(y) vs. y.

distribution function is available, a computational procedure or special graph paper is used to perform the transformation. As with the logit, if the data does appear to be the graph of the CDF of a normal distribution, then probit(y) is a linear function of x,

$$\text{probit}(y) = \alpha + \beta x,$$

where $\alpha = 5 - (\mu/\sigma)$ and $\beta = 1/\sigma$. These parameters are determined by linear regression. If the plot of probit(y) vs. x is not a straight line, the data is not the CDF of a normal distribution.

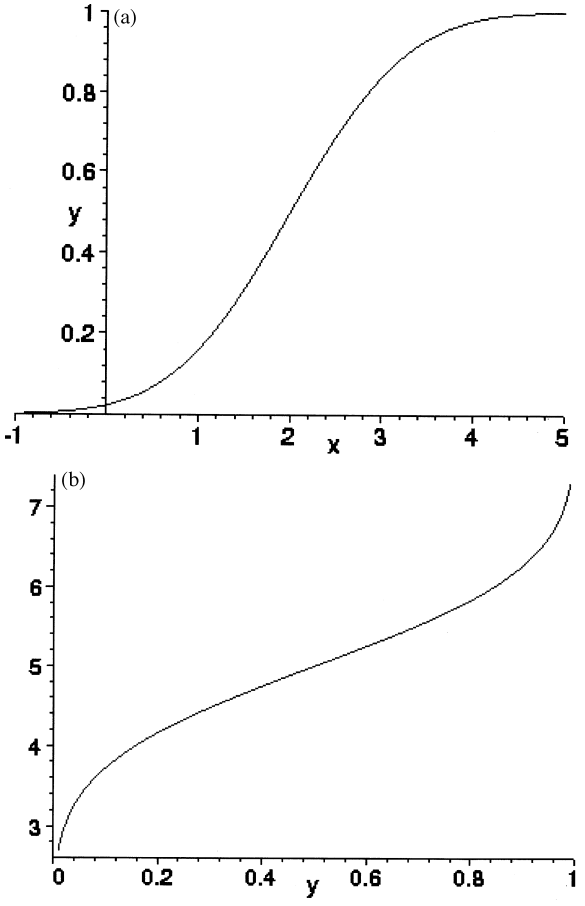

Fig. 4. (a) $y = F(x)$ a typical sygmoidal curve before the probit transfromation (The CDF for a normal distribution which has a mean of 2 and S.D. = 1). (b) The function, $\text{probit}(y) = \Phi^{-1}(y) + 5$, where $\Phi(z)$ is the CDF for the standard normal distribution (z-scores).

A plot of a typical sigmoidal function of this type and probit(y) are presented in Fig. 4a,b.

The use of probit in the setting of immunological methods, and software to make the calculation of the transformation and the regression more automatic can be seen in Smith et al. (1977), Sette et al. (1986), Wallis (1991), and Bailey et al. (1992). Logit has been used in Kobayashi et al. (1978). Fey (1981) and Reif and Robinson (1975) also addresses the problem of fitting sigmoidal curves. See also two papers by Stein et al. (1977) and Alvord and Rossio (1993) involving bioassay, in which these same problems arise.

2.2. The precipitation reaction

The interaction of some antigens and antisera raised against that antigen can result in a precipitate, first observed in 1897 with culture filtrates from the plague bacilli. The understanding of the nature of the reaction, particularly the observation that when the antigen and antibody are in 'equivalence', the largest amount of precipitate results (Fig. 5), turned out to be a difficult theoretical problem (reviewed in Wells, 1925) as even the chemical nature of the interaction of antigens and antibodies was not established at that time. Quantitative studies by Heidelberger which lead to the use of the chemical law of mass action (Heidelberger and Kendall, 1935; Heidelberger, 1939) and the lattice model of Marrack (1934) established that the antibody molecule was at least bivalent by showing that the mole ratio of the precipitate can change as antigen is added. Their work gave quantitative and geometric interpretations for the features of the data.

In the case of Heidelberger's approach, the parameters resulting from the model description have had lasting meaning. For a small monovalent ligand, L, let S be a representative antibody binding site which could bind to the ligand. Consider the reversible reaction

$$S + L \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} SL$$

Using the law of mass action, a differential equation which describes the rate of change of

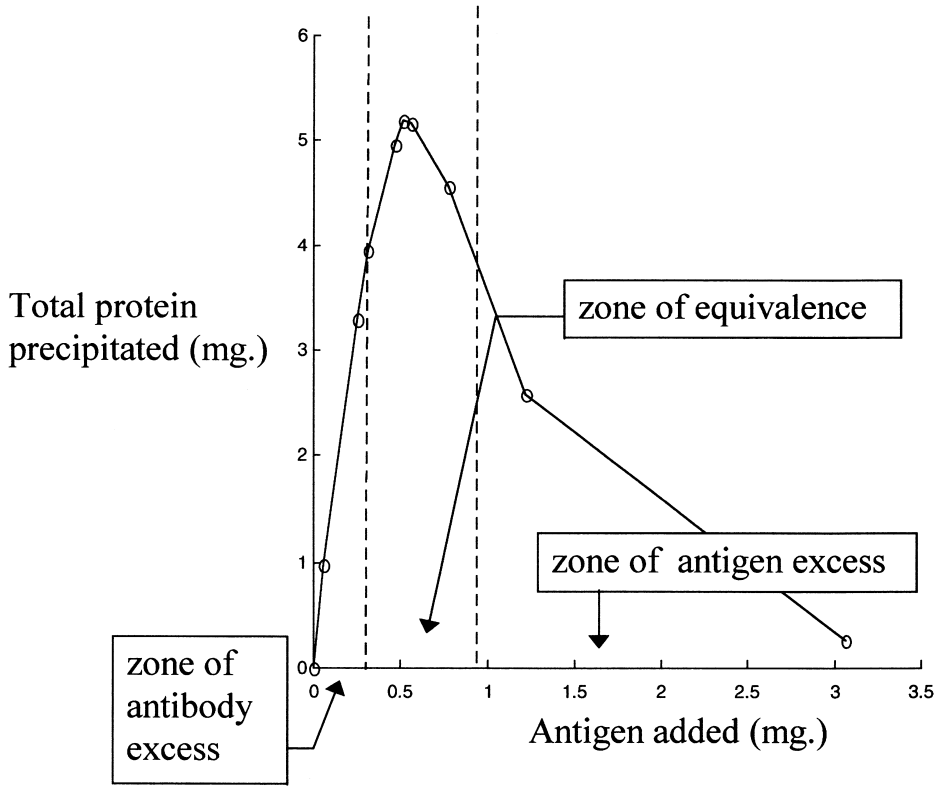

Fig. 5. Drawn from data from Heidelberger and Kendall (1935), illustrating the relationship between an antigen added [in this case alum-precipitated crystallized chicken ovalbumin (EAc)] and precipitate. The amount of antibody present is the same in each trial. The tubes corresponding to small amounts of added antigen were shown to contain unprecipitated antibody and little unprecipitated antigen (antibody in excess) while those tubes with larger amounts of added antigen contained little unprecipitated antibody and increasing amounts of unprecipitated antigen (antigen excess). The middle region, which corresponds to the largest amount of precipitate is the 'equivalence' zone.

the concentration of the SL complex over time in a well-mixed compartment of ligands and binding sites is

$$\frac{\mathrm{d}[SL]}{\mathrm{d}t} = k_1[S][L] - k_{-1}[SL],$$

where $[S]$ is the free antibody binding site concentration, $[L]$ is the free (unbound) ligand concentration, and $[SL]$ is the concentration of the complex. Assuming that this reaction reaches equilibrium rapidly, after negligible time the difference on the right hand side is zero, and a ratio of the reaction rates can be computed,

$$\frac{k_1}{k_{-1}} = \frac{[SL]}{[S][L]}. \qquad (4)$$

This ratio, usually assigned the letter K, is the *intrinsic association constant* or the *intrinsic affinity* of a binding site for the ligand. This is the simplest interaction possible. If the ligand is not small, interference with other binding sites on a single antibody molecule can result. In addition, the number of antibody binding sites on a single molecule — the valence — (circulating IgM vs. IgG, for instance) also changes the 'observed' affinity, the avidity. A complete discussion of antibody–antigen reactions and their dynamics can be found in Fazekas de St. Groth (1979) and Steward and Steensgaard (1983). A concise presentation can be found in Eisen (1980).

For multivalent ligands, the reaction can proceed further, as the SL complex can combine

with additional antibody binding sites, ligands, and complexes, leading in some cases to the insoluble precipitate — the *precipitin reaction*. If the antigen is on a bacterial cell wall or a red blood cell, *agglutination* can result. If the antibody is on a surface, as an antibody receptor on a B lymphocyte (or IgE bound to an Fc receptor on a mast cell), the interaction of an antibody and antigen (or allergen) results in cross-linking, leading to patching and possibly stimulation (or histamine release). These aspects have a large literature — Bell (1974), DeLisi (1976) and Bell and Perelson (1978) serve as an introduction. A recent paper which illustrates the interaction between the curves which reflect binding as a function of an antigen dose and theoretical models can be found in Sulzer and Perelson (1997). An interesting liposome-based immunoassay where the distribution of valences was studied is found in Waite and Chang (1988).

The literature of the use of computational models in describing aspects of the immune response in vivo and in theoretical immunology is large, represented by the reviews, monographs, and proceedings (Bell et al., 1978; Bruni et al., 1979; Merrill, 1980; Marchuk and Belykh, 1982; Marchuk, 1983; Hoffmann and Hraba, 1986; Perelson, 1988, 1992; Prikrylova et al., 1992). These efforts will not be discussed here, concentrating instead on the role of computational models in the laboratory. Also, the statistical analysis of immunological data generally follows standard biostatistical texts. One review of some useful statistical techniques specific for immunology can be found in Piazza (1979).

3. Modern contributions of computational models to immunological methods

In this section, areas of immunological methods in which computational models have and are playing a role are briefly discussed.

3.1. Qualtity (affinity) of an antibody

The antibody involved in a binding reaction with ligand, as in the precipitin reaction discussion above, the affinity can be determined through a graphical process. Starting with Eq. (4)

$$\frac{k_1}{k_{-1}} = K = \frac{[SL]}{[S][L]},$$

dividing both numerator and denominator by the total antibody concentration

$$K = \frac{\frac{[SL]}{[Ab]}}{\left(\frac{[S]}{[Ab]}\right)[L]} = \frac{r}{(n-r)c} = \frac{1}{n-r}\left(\frac{r}{c}\right) \qquad (5)$$

where c is the free ligand concentration ($[L]$), r is number of ligand molecules bound per antibody molecule (when the free ligand concentration is c), and n is the antibody valence (and thus $n-r$ is the number of available antibody binding sites per antibody molecule). Note that r depends (or is a function of) c. Multiplying through Eq. (5) by $n-r$, one obtains the Scatchard equation (Scatchard, 1949),

$$\frac{r}{c} = Kn - Kr. \qquad (6)$$

Plotting r/c vs. r should result in a straight line of slope $-K$. This result requires that all antibody binding sites are identical and they are independent (binding one site on a molecule does not affect the binding at another site on the same molecule). The average affinity, K_0, is the reciprocal of the free ligand concentration, c, which results in an r value which is $n/2$ (half of the antibody sites occupied). The use of the Scatchard plot is illustrated in Porstmann et al. (1984). Other rearrangements of Eq. (5) are also used to compute these parameters. The most important is the Langmuir plot, $1/r$ vs. $1/c$, where the slope of the line is $1/nK$ and intercept $1/n$. Each transformation, mathematically, results in the same result, however, statistical properties of the transformed data often make one transformation a better choice for linear regression. A derivation of a similar nature using an enzyme–kinetic approach is found in Hoylaerts et al. (1990). Fjeld

and Skretting (1992) discuss simultaneous computation of the kinetic parameters. Cumme et al. (1990) discuss computations in the case of monoclonal antibodies to enzymes (see also Strike et al., 1979; Jacobsen et al., 1982; Pellequer and Van Regenmortel, 1993).

The recognition that the Scatchard plot is often not linear (e.g. Eisen, 1980), suggests that the antibody is generally a heterogeneous mixture with respect to its affinity, unless it is an monoclonal antibody. The heterogeneity of the mixture can be quantified through the use of a parametric expression for the affinity distribution. A common approach to the determination of the nature of parameters which characterize antibody heterogeneity is through the Sips distribution (Sips, 1948). This distribution depends on specifying two parameters, K_0 and α. The parameter K_0 is the average affinity (the median of the distribution) while α, with values between 0 and 1, determines the S.D. of the distribution (Bruni et al., 1976),

$$\sigma = \pi\sqrt{\frac{1-\alpha^2}{3\alpha^2}}\,.$$

If a pool of an antibody was distributed as a Sips distribution, for α near 1, the antibody is nearly homogeneous, while α near 0 gives a very broad distribution. The functional form for the Sips distribution is (Bruni et al., 1976)

$$p(K) = \frac{1}{2\pi K} \times \frac{\sin(\pi\alpha)}{\cosh(\alpha(\ln(K) - \ln(K_0))) + \cos(\pi\alpha)}.$$

The distribution is approximately symmetric, with a peak at K_0 and a very narrow spike near 0 as illustrated in Fig. 6a for α near 1. As α decreases, the spike near 0 grows until the distribution becomes a decreasing function of K as illustrated in Fig. 6b.

The Sips approach is made attractive by the existence of an easy test for the appropriateness of the assumption, and the computation of the parameters. If the plot $\log(r)/(n-r)$ is plotted vs. $\log(c)$, and the affinity distribution is a Sips

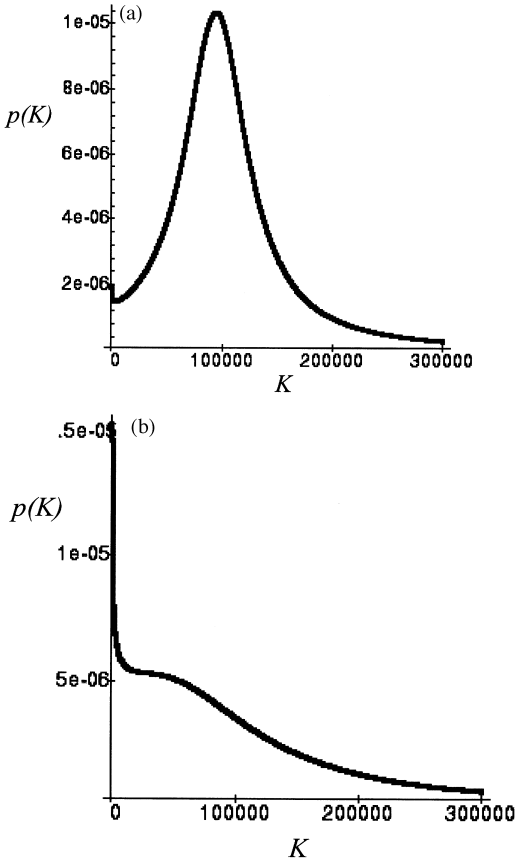

Fig. 6. (a) A typical Sips distribution with α near 1. For this plot, $K_0 = 10^5$ and $\alpha = 0.9$. (b) A typical Sips distribution with small α. Here $K_0 = 10^5$ and $\alpha = 0.6$.

distribution, then the plot (the Sips plot) will be linear with slope α,

$$\log\left(\frac{r}{n-r}\right) = \text{intercept} + \alpha\log(c). \tag{7}$$

Moreover, the *intercept* can be computed when the free ligand concentration c is chosen to result in $r = n/2$. Then Eq. (7) becomes

$$\log\left(\frac{r}{n-r}\right) = \log\left(\frac{n/2}{n-(n/2)}\right) = \log(1) = 0$$
$$= \text{intercept} + \alpha\log(c),$$

or intercept $= -\alpha\log(c) = \alpha\log(K_0)$ where K_0 is the average affinity above. As a result, through

the process of linear regression, a fit to a Sips distribution can be quickly made, with the heterogeneity and the average affinity natural parameters.

If the Sips plot is not linear, as is often the case, other methods are necessary to determine the affinity distribution. A method that results in a four parameter distribution (which is generally bimodal) is presented in Bruni et al. (1976). Such a parametric approach is desired if one is describing the evolution of affinities in a primary humoral response. If the problem is a static one, a non-parametric approach is usually preferred, such as in Yuryev (1991) in documentation which accompanies his software. Also note two simulation studies Chiecchio et al. (1992) and McGuinness et al. (1997) and Underwood (1985).

3.2. Agglutination and hemagglutination

Adding an appropriate antibody can cross-link antigens on cell surfaces or bacterial cell walls. The clumping which results is called agglutination. The reaction is used to identify bacteria, blood cell types, isotypes of an antibody (Aubert et al., 1995) and to quantify an antibody in sera (Ambrose and Donner, 1973), through measuring the degree of agglutination corresponding to a serial dilution of an antibody. Hemagglutination is the name given to the reaction when red blood cells are the target of agglutination. A variation of the assay, agglutination inhibition, is used to measure hormones, with an idea of inhibition of the agglutination by adding hormone (from sera) not bound to red cells. See the discussion on radioimmunoassays for a similar approach. A statistical procedure for these tests can be found in Schuurs et al. (1972).

3.3. Immunoassays — quantification of antibodies or ligands

Immunoassays are a special case of assays involving the binding of two substances — one that is to be quantified or detected (the analyte), and the other substance one that specifically binds the substance of interest. In immunoassay, one of the substances will be an antibody. The specific type of immunoassay is dictated by the goal of the assay and the manner in which the results are obtained. A concise description of the many approaches to immunoassays (and how they are named) can be found in Ekins (1992).

If the binding can be directly observed as a precipitate, the analyte can be directly quantified through gel precipitation methods, such as double immunodiffusion or through immunoelectrophoresis. Sensitivity of an assay can often be increased through labels or indicators. These include red cells (with an antigen on the surface) with agglutination of the cells if an antibody to the antigen is present, radiolabels (e.g. radioimmunoassays, RIA; enzymes (e.g. enzyme-linked immunsorbant assay, ELISA); and fluorophors (e.g. fluoroimmunoassay, FIA). With each label there are instances when the antibody or the antigen is labeled, and special techniques (and abbreviations) for each. For the purpose of this review, we start with a historical view.

3.4. The Farr assay

The Farr assay (Farr, 1958) quantifies the antibody to a given antigen in sera through precipitation of Ab–Ag complexes through the addition of ammonium sulfate at high concentration. A radio-labeled antigen allows the quick determination of the proportion of the label (and thus antigen) in the precipitate. The method is often employed in the measurement of anti-DNA antibody. The Ag-binding capacity (ABC) is determined through serial dilution of the sera. The result of the analysis usually reports the ABC as the reciprocal of the serum dilution at which some predetermined fraction, say 50%, of the label is in the precipitate. The relationship between ABC at a particular antigen concentration, and the actual titer and distribution of affinities requires a model. Moreover, errors in the assay, with respect to the low importance of a low affinity antibody in the index, and the exaggerated importance of any high-affinity antibody makes it necessary that any results be carefully examined (Kim et al., 1975; Aarden et al., 1976; Koch and Oratore, 1978).

3.5. Radioimmunoassays

Radioimmunoassays date from Yalow and Berson (1960) who designed the method to study the fate of insulin in diabetics. They found that diabetics had anti-insulin antibodies which affected the ability of the insulin from ever leaving the capillaries. This was discovered after injecting radiolabeled insulin in individual patients. This 'accidental' finding led to the in vitro assay through utilizing the competitive inhibition by unlabeled ligand for the binding of labeled ligand with an antibody. The method can be used to measure the concentration of any ligand for which a specific antibody is available (primarily hormones). To a fixed concentration of the specific antibody and radiolabeled ligand, several different concentrations of unlabeled ligand are added. With each concentration of unlabeled ligand added, the ratio of bound labeled to free labeled ligand is determined — several methods being available. A calibration curve consisting of a plot of the ratio of bound-labeled to free-labeled ligand vs. the concentration of unlabeled ligand added is thus constructed. To an unknown concentration of the ligand, the antibody and labeled ligand is added and the above ratio computed. Using the calibration curve, the unknown ligand concentration is determined.

Modeling aspects enter this class of assays in a number of different ways. The simplest is in the determination of the calibration curve (which is not linear) and its use. The process is called 'inverse non-linear regression', and determination of confidence intervals for the concentrations requires care. An automated procedure is presented in Ventura et al. (1985). Variations in the procedure also permits the determination of affinities in Gaze et al. (1973), Van Heyningen et al. (1983), and Larsson and Axelsson (1991).

The dynamics of the binding of the ligand to the antibody, and its equilibrium behavior can be determined. The rate of change of the concentration of the *Ab-ligand* complexes (bound ligand) with respect to time. Let $[Ab]$, L, L^* be the concentrations of anti-ligand antibodies, unlabeled ligands, and labeled ligands, respectively. The first and third are constant throughout the assay while several values of L will be used. Then, assuming that the binding satisfies the law of mass action (the mixture is well-mixed) and that the kinetics of binding to the labeled and unlabeled ligand are identical (and the rate constants k_1 and k_{-1} are as before), then

$$\begin{aligned}\frac{\mathrm{d}[Ab \sim \text{ligand}]}{\mathrm{d}t} &= k_1([Ab] - [Ab \sim \text{ligand}]) \\ &\quad \times (L + L^* - [Ab \sim \text{ligand}]) \\ &\quad - k_{-1}[Ab \sim \text{ligand}] \end{aligned} \tag{8}$$

At equilibrium, the derivative is 0, and as a result,

$$\begin{aligned} K([Ab] - (\text{bound ligand})) &= K([Ab] - [Ab \sim \text{ligand}]) \\ &= \frac{[Ab \sim \text{ligand}]}{L + L^* - [Ab \sim \text{ligand}]} \\ &= \frac{\text{bound ligand}}{\text{free ligand}} \\ &= \frac{\text{bound labeled ligand}}{\text{free labeled ligand}} \end{aligned} \tag{9}$$

The bound ligand concentration depends on the quantity L of unlabeled ligand added, making Eq. (9) an implicit relationship for the bound ligand as a function of L. Using the quadratic formula, this functional relationship can be specified. This relationship is plotted in Fig. 7 for a particular values of Ab, L^*, and K. This is the radioimmunoassay calibration curve. The functional relationship and aspects of the plots can be used to compute other quantities of interest. Examples of this work can be found in Niederer (1974), Revoltella et al. (1974), Van Munster et al. (1978), Muller (1980), Stanley and Guilbert (1981), Antoni and Mariani (1985), Boscato et al. (1989), Larsson (1997), Massino et al. (1997), Lövgren et al. (1997), and O'Connor and Gosling (1997).

3.6. Precipitation reaction in gels

Double immunodiffusion was developed to quantify either an antibody or ligand through the position of precipitation bands (region of equiva-

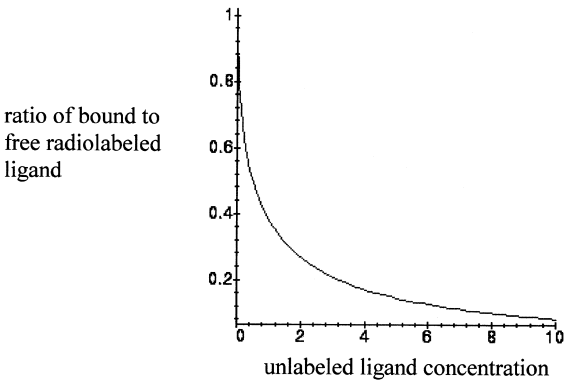

Fig. 7. Typical calibration curve for a radioimmunoassay determined by the relationship in Eq. (9).

lence) in a gel. There are several methods and variations, each involving wells or regions of antibody and ligand, sometimes several of them, with the gel serving as the medium for the diffusion outward from the wells. A concentration profile is determined through a model of the diffusion of each of the reactants through the gel, so that knowing one initial concentration, and the position of the precipitate, can determine the concentration of the unknown concentration. An illustration of the reaction of two reactants along with their concentration profiles is given below in Fig. 8 for the case of estimating the antibody concentration.

A variation of this technique, radial immunodiffusion (RID) computes concentration of an antibody (or antigen) by measuring the diameter of a ring of precipitation formed from the interaction between the substance of interest, and a gel-immobilized antigen (or antibody). The diameter measurement permits the computation of the area inside the ring which is functionally related to concentration.

Utilizing electrophoresis to first separate components of a mixture in a gel, then visualizing precipitation bands corresponding to the components through a process similar to double diffusion (with several specific antibodies) is the basis of immunoelectrophoresis. The reliable quantification of the results has been reviewed in Axelsen and Bock (1972), with specific examples in Birkmeyer et al. (1981). In Chen et al. (1994), a simulation of the melting of DNA along with temperature gradient electrophoresis is used.

3.7. Nephelometry

In nephelometry, estimation of antibody or antigen concentrations is accomplished through the use of the light-scattering properties of complexes. It is a modification of the precipitin reaction in that the measurements of increased scattering due to complexes is done in the antibody excess zone. Repeated measurements are made

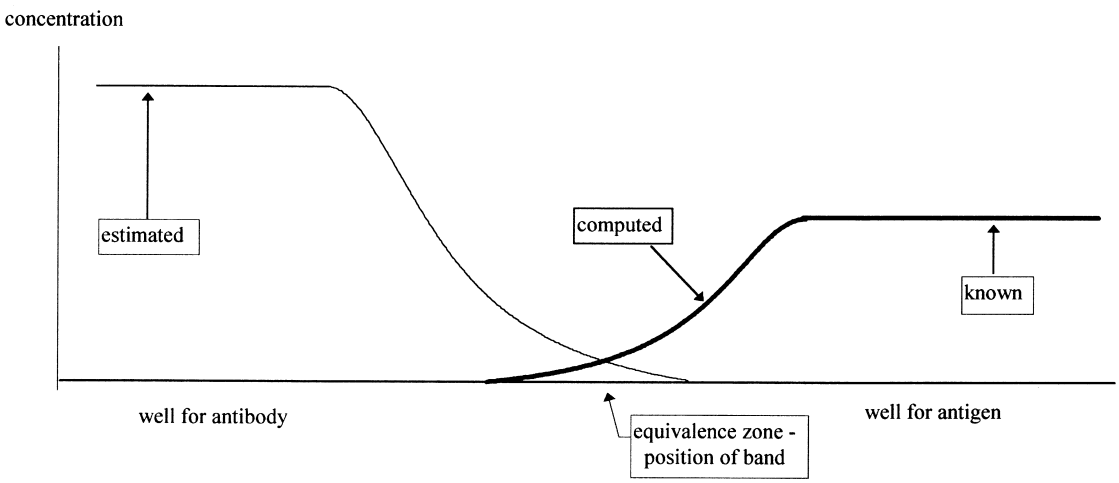

Fig. 8. The estimation of antibody concentration through immunodiffusion.

as the antigen (or antibody) is titrated — using a calibration curve to estimate the concentration of interest. Deverill and Reeves (1980) reviews applications of the measurement of turbidity in this immunological setting. The basic mathematical problem is that the relationship between absorbance and antigen concentration is non-linear (Foster and Ledue, 1986). An example can be found in Cambiaso et al. (1974).

3.8. Immunofluorescence and fluorescent labeling of cells

Antigens or antibodies can be given fluorescent labels, with the objective generally being to localize or quantify the binding of an antibody to specific sites in tissues or on cells. The assay is an immunofluorescence assay if a labeled antibody is used and fluoroimmunoassay if an antigen is labeled. In addition, cells are given fluorescent labels by antibodies specific for distinguishing markers before sorting these cells in a fluorescence activated cell sorter (FACS). A similar process for the fluorescent microscope is also used to localize markers, their density, and distribution on cells of interest.

To measure the concentration of an antibody in serum to a given antigen, often in frozen sections or tissues, a fluorescein–conjugated anti-Ig antibody with the ability to bind all antibodies of interest is used in the immunofluorescence assay. After incubation of the serum with the tissue, gentle washing leaves antibodies which binds to the tissue. Fluorescent-tagged anti-Ig is added, binding to all bound antibodies. The level of fluorescence observed is often proportional to the quantity of the antibody which has bound to the tissue. This is the 'indirect' procedure which is more sensitive than the 'direct' procedure in which all antibodies in the test sera is labeled. As a quantitative assay, there are difficulties with the indirect method as several labeled antibodies can bind to a single antibody of interest, and as a result, the amount of fluorescence in a sample depends on several parameters. This, and related problems are studied in Coleman et al. (1972) and Jobbágy and Jobbágy (1972a,b). Quantitative aspects of the fluorescent labeling of cells for fluorescent microscopy can be found in Dormer et al. (1981), Pachmann and Killander (1976), and Hesford et al. (1987). Aspects of fluorescent labeling in flow cytometry is seen in the review of Rolland et al. (1985) and in Bardsley et al. (1992).

3.9. Enzyme immunoassay and ELISA

Enzyme immunoassay (EIA) was introduced in by Engvall and Perlmann (1972), for applications where RIA would also be an option. This general class of assays can involve the detection of an antibody or an antigen of interest. The basic idea is to bind an enzyme-conjugated anti-Ig to an antigen–antibody complex. Adding the substrate for the enzyme can produce a color change or fluorescence in the product which is easily quantified — the magnitude of the color change or the fluorescence is related to the amount of the complexes present, although difficulties can be present in interpretation (reviewed in Pick and Mizel, 1981; Stemshorn et al., 1983; Beatty et al., 1987; Francois-Gerard et al., 1988; Ehle et al., 1989; Pesce and Michael, 1992; Herraez, 1993). Enzyme-linked immunosorbent assays (ELISA) refers to the assay involving a solid-phase antigen. As this assay is often done in microtiter plates, much of the work involving computational models involves the methodology in dealing with the data in that form, and with enhancements to this assay (e.g. Canellas and Karu, 1981; Richardson et al., 1983; Slezak et al., 1983; Caulfield and Shaffer, 1984; Franco et al., 1984; Slade et al., 1986; Zrein et al., 1986; Mixter et al., 1986; Karpinski et al., 1987; La Belle, 1987; Gigase et al., 1988; Huet et al., 1990; Raghava, 1992; Glaser, 1993a,b; Tremain 1993; Reiken, 1994; Fucks et al., 1995; Reizenstien et al., 1995; Stevens and Kelso, 1995; Iznaga Escobar et al., 1996). Problems with affinity measurements in ELISA are discussed in Underwood (1993); a discussion of the statistical framework for ELISA assays can be found in Bunch et al. (1990), Sittampalam et al. (1996), and McGuinness et al. (1997).

The oldest form of enzyme immunoassay is hemolysis, the complement-mediated lysis of red blood cells discussed previously. Lysis of the cells indicates the presence of a complement fixing

antibody which can bind to an antigen on the surface of the red cells. Using erythrocytes as indicators in assays, such as the Jerne plaque assay (Jerne and Nordin, 1963), allows both an indication of the number of antibodies producing cells and an estimate of the rate of antibody production and affinity. These last results require computational models of the assay. DeLisi and Bell (1974) and Jerne et al. (1974) present general theoretical results. Sette et al. (1988) present software for analysis of modulation of cellular responses through the use of plaques. Esrig et al. (1977) present a computational approach suitable for the laboratory.

3.10. Cellular assays

Computational modeled applied to cellular assays both developed later, and generally has a different nature than that discussed thus far. More of the work involves statistical models, and mathematical models and simulations that do appear tend to be more sophisticated. Given the nature of this review, the discussion area of each will be brief, with one exception.

3.11. Cytotoxicity assays

There are several approaches to quantitatively describing the action of a cytotoxic cell population on a 'target' cell — usually a susceptible cell line. One which has turned out to be applicable to many cell types (cytotoxic T lymphocytes and NK cells in particular) is to use an analogy with classical enzyme kinetics (Michaelis and Menten, 1913) where the cytotoxic cells play the role of an enzyme, the product being eventual target cell lysis. Lysis can be noted either through the release of a radioactive label or through non-isotopic markers (such as in a colorimetric assay). The 'substrate' in the enzyme–kinetic analogy is the target cell — usually supplied in excess in the assay (Dunkley et al., 1974; Miller and Dunkley, 1974; Thorn and Henney, 1976; Zeijlemaker et al., 1977; Callewaert et al., 1978). The advantage of using this analogy is that familiar transformations, such as the double reciprocal Lineweaver–Burk plot, can be directly applied, along with extensions to this formalism to include inhibition and other factors, and improvements in parameter estimation (Callewaert et al., 1982; Merrill, 1982; Merrill and Sathananthan, 1986; Garcia-Penarrubia and Bankhurst, 1989; Garcia-Penarrubia et al., 1989, 1992, 1995; Galvez et al., 1994). Other approaches to quantifying cytotoxicity can be found in Chalmers et al. (1982), Crispe and Gascoigne (1983), Hogan and Evans (1984), Bol et al. (1986), Hudig et al. (1988), and Sheeran et al. (1988). Rodgers et al. (1992) used a colorimetric assay to identify CTL determinants.

3.12. Migration, migration inhibition, phagocytosis assays

The description of the movement of cells has two basic techniques, one using a micropore filter and the other, the 'under agarose' method. Descriptions of these methods can be found in Maderazo and Ward (1986). The basic quantities involve describing the movement (chemotaxis), chemoattraction, chemokinesis, and the inhibition of these quantities. The work in this area is by nature computational as even the parameters of movement and the nature (randomness or purposeful) needs to be described in some way. The following papers deal with the many different approaches to these related problems: De Halleux and Deckus (1975), Fenton and Taylor (1975), Watanuki and Haga (1977), McDaniel et al., 1978, Weese et al. (1978), Moss et al. (1979), Turner (1979), Axelsson et al. (1981), Lauffenburger and Zigmond (1981), Repo et al. (1981), Fordham et al. (1982), Hamblin et al. (1982), Rhodes (1982), Stickle et al. (1984), Minkin et al. (1985), Pedersen et al. (1988), Buettner et al. (1989), Jensen and Kharazmi (1991) (using image analysis), Haddox et al. (1991, 1994), and Azzara et al. (1992). Assays of phagocytosis of many of these same cells is described in MacFarlane and Herzberg (1984) and Saad et al. (1985).

3.13. Limiting dilution assays

Limiting dilution assays date from early bacte-

riology in the late 19th century, when a source material was diluted until either one or no bacteria were present. It was used as a method of purification of a bacterial sample. In immunology, this class of assays can be used in a very quantitative way, both to quantify the frequency of particular cell types in a sample and to identify the number of different cells responsible for an observed event. Analysis of the results is generally assumed to follow Poisson statistics, and that approach is standardized through the analysis of graphs on semilogarithmic paper. A monograph on the subject as Lefkovits and Waldmann (1979), and many reviews, such as Fazekas de St. Groth (1982), are readily available.

When many small aliquots of cells are drawn from the same source, and there is a rare cell type in that source the number of rare cells in the wells will follow a Poisson distribution (which approximates a binomial distribution in this case). In that way, the expected number of aliquots containing 0, 1, 2 and more cells of the rare type is known, and depends on the frequency of this rare type. From the Poisson distribution, the fraction of aliquots with n of the rare cells should be approximately

$$P(n) = \frac{\lambda^n}{n!} e^{-\lambda}$$

where λ is the average number of rare cells per aliquot. Put into microwells, the probability that an aliquot contains no rare cells (is 'unresponsive' in most assays) at each dilution is $P(0) = e^{-\lambda}$. With each dilution this average number of rare cells in the cultures is reduced. Taking the natural (base e) logarithm of the expression for $P(0)$, we find that

$$\ln(P(0)) = \ln(e^{-\lambda}) = -\lambda. \quad (10)$$

As a result, the negative logarithm of the fraction of non-responsive wells is an estimate for λ at each dilution. Also, since dilution should not change the fraction of rare to other cells in the well, merely reduce the overall number of cells,

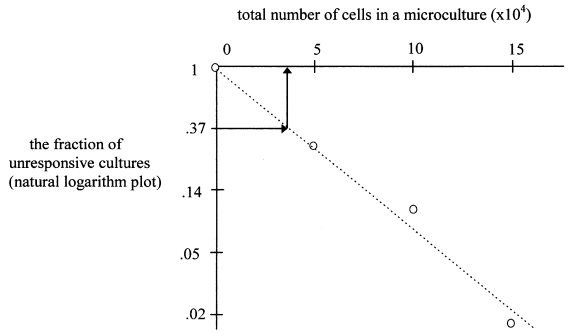

Fig. 9. The linear relationship between the number of cells in culture and the fraction of non-responsive cultures plotted on a (natural) semilog plot, so that no computations of the logs are required. The position on the line corresponding to 0.37, $\log_e 0.37 = 1$, inidcates the dilution when the average number of rare cells is 1 (the fraction of non-responsive cultures is 0.37).

the average number of rare cells per well should be

$$\lambda = (\text{fraction of rare cells in the original sample}) \cdot (\text{number of cells in the dilution}), \quad (11)$$

or λ is a linear function of the number of cells in the dilution. Putting Eq. (10) and Eq. (11) together suggests that the negative logarithm of the fraction of non-responsive wells ($\approx \lambda$) is proportional to the number of cells in a dilution. This is illustrated in Fig. 9.

This assay has many other aspects which are discussed in the above references and in the following papers Taswell (1984), Lietzke and Unsicker (1985), Koziol et al. (1987), Strijbosch et al. (1987), Koziol (1988), Underwood and Bean (1988), Burleson et al. (1993), Bonnefoix and Sotto (1994), Bonnefoix et al. (1996), and Palenzuela et al. (1997). Of special note is the use of a proliferation assay to compute a responsive-cell frequency in Broman et al. (1996).

3.14. Basophil degranulation

The release of histamine can be used as an indication that some event has taken place, or it can be quantified in the study of the binding of an

allergen to IgE on armed basophils or mast cells. There is a quite an extensive literature of these problems and their quantitative description, reviewed in Dembo and Goldstein (1980). Specific examples can be found in MacGlashan et al. (1985) and Sainte-Laudy (1987). A related but different assay is that of RAST, the radioallergosorbent test, described in Bongrand et al. (1976).

3.15. Proliferation assays

Proliferation of lymphocytes in response to a mitogen, antigen, or as modified by immune modulators all involve assays of proliferation. The standard approach is to measure incorporation of [^{3}H]-thymidine in dividing cells. The mechanics of the assay and the treatment of the CPM data from the gamma counter is described in Dei and Urbano (1977). Description of the results using models can be seen in Gibbs et al. (1979), Jensen et al. (1981), Cason et al. (1987), Kenter and Watson (1987), and Niks et al. (1990). Svirshchevskaya et al. (1993) utilize enzyme kinetic-like equations to study IL-2-induced proliferation. Bennett and Riley (1992) discuss the difficulties in developing binary data — responders and non-responders in a lymphocyte proliferation assay.

3.16. Miscellaneous assays

Immunology laboratories perform other assays which do not fit comfortably within the framework given above. For instance Wei et al. (1975) discuss liposome spin immunoassay for detecting lipid substances in aqueous media. Johns and Stanworth (1976) characterize the nature of immunoglobulin complexes moving at the same speed in sucrose density gradients while Stewart and Johns (1976) construct a relationship between the sedimentation coefficient and molecular weight. Brown et al. (1979) provide an automated method for quantifying E-rosettes using a particle size analyser while Wenger et al. (1982) apply similar instumentation to evaluate the size distribution of cells. Cheewatrakoolpong et al. (1983) offer software to evaluate the clearance kinetics of bacteria in mice.

3.17. Other general topics

The following topics involve either general computational methods which could be applied to a number of different assays, or molecular methods whose applicability is also central to several immunological methods.

3.18. Computational approaches to data and experimental design and data storage

Because of the complexity and amount of data that can be collected, some ideas on how the data will be stored, coded, and analyzed is necessary. The following papers and software deal with that problem in a number of different settings Franklin (1982), Hooton and Paetkau (1986), Watson et al. (1988), Stone et al. (1991), Delaage et al. (1992), Siman (1992), Bishop et al. (1993) and Greiner (1995, 1996).

3.19. Gene sequencing and libraries

The ability to sequence DNA and RNA, along with the use of computers to search and manage libraries of sequences has brought about a revolution in the molecular side of immunology. The development of the algorithms necessary to knit together the fragments resulting from the sequencing process has developed in parallel to the automated sequencing tools. Mathematical introductions to this topic can be found in Lander and Waterman (1995), Waterman (1995), Speed and Waterman (1996), and Setubal and Meidania (1996). The searching for homologies in sequences also involves a computational model, in that ‘best fit’ between two sequences involves both a difficult optimization problem and some assumptions concerning conserved regions and those which may be variable, for instance. The fragments that result from the restriction enzymes can also yield important immunological information, as in Liu et al. (1995).

3.20. Structures of proteins

The ability to determine the amino acid sequences of proteins has brought with it the prob-

lem of determining the shape (secondary and tertiary structure) of these proteins, which may be receptors, antibodies, or ligands. The mathematical problem of finding the shape which minimizes the free energy of the molecule is a difficult problem (Merz and Le Grand, 1994; Cohen, 1995; Fraga et al., 1995; Kolinski and Skolnick, 1996). In addition, this predicted structure will usually depend on the physiological conditions, and there is no guarantee that the predicted conformation will closely relate to the actual structure, as there are often several possible confirmations which are local minima of the free energy (Novotny et al., 1988). Also, there is no guarantee that the physiologically most important confirmation is the confirmation predicted by the global minimum value of the free energy. In a few cases (e.g. Huang et al., 1990; Bajorath et al., 1997) aspects of the true structure and the prediction can be compared. Although they are rarely identical, often important features are present in both. This is an area which has benefited from recent advances in computing and visualization, along with the improvements to the mathematical algorithms. Using hydrophobicity to identify antigenic determinants has been reviewed in Hopp (1986). Primary structure (sequences) are searched for MHC motifs in protein sequences in Jones and Wei (1995).

3.21. PCR — the polymerase chain reaction

The polymerase chain reaction serves as a tool for amplification of sequences of interest to enable further procedures and analysis, detection of the presence of rare sequences, and in quantification through a number of methods. The idea of amplifying DNA in a test tube dates from 1971, but it was not until Mullis's use of the thermostable Taq polymerase in 1987 that the method was seen as practical, cheap and essential. An early readable review of the use of PCR in molecular immunology is Thiesen et al. (1990). Models enter this arena primarily in the attempt to quantify the number of target sequences in the original cycle from knowing the amplified number after cycling. The earliest work in that area is Harris (1992), which involved a stochastic model and inverse prediction. More recent efforts can be seen in Melby et al. (1993) and Connolly et al. (1995).

3.22. Image enhancement and reconstruction, localization

The successful application of computers to enhance optical instruments from microscopes to scanners is the result of the application of computational models. The converting of the true image to a sampled or digitized one involves the loss of information. The reconstruction of the enhanced image is based on algorithms applied to the digitized data along with assumptions concerning the nature of the reconstructed image. A very fine review has just appeared, Sabri et al. (1997), and as a result, this section will be brief. The development of imaging systems can be seen in several different applications, including Cushley et al. (1983), Gershwin and Olsen (1985), Rogers (1985), Maly et al. (1989), Munn et al. (1993), and von Olleschik-Elbheim et al. (1996).

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