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Improving methods of assessing natural killer cell cytotoxicity

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Abstract
Natural killer (NK) cells are a class of lymphocytes important in immune resistance to viral and other serious diseases. The cytotoxic function, or 'killing activity' of NK cells has become important in studies of the effects of stress and other psychosocial factors on physical health. Unfortunately, research on NK cell function has been plagued by discrepancies in the methods of interpreting NK cytotoxicity data. We briefly review some of the variations in measuring NK cell activity and present a new model for interpreting these results, introducing maximal target cell lysis (A) and the slope of the cytolytic curve (k) as parameters that attempt to make full use of the information and the statistical power in NK cell cytotoxicity data. Examples of these interpretation methods are presented using NK cytotoxicity data from a group of metastatic breast cancer patients. This approach will be useful in applications of NK cell measurement in psychoneuroimmunology research. Copyright © 2006 John Wiley & Sons, Ltd.

Key words: natural killer, cytotoxicity, psychoneuroimmunology, cancer

Introduction

Studies of natural killer (NK) cells, a front-line immune defence against tumours and some viruses (Whiteside and Herberman, 1995; Moretta et al., 2002; Smyth et al., 2005) have shown that psychosocial factors are linked with immune function. Morphologically identified as large granular lymphocytes, mature circulating NK cells have cell surface antigens CD16 and CD56, but not CD3. Natural killer cells comprise only a small proportion (5% to 15%) of the lymphocytes in peripheral blood (Whiteside and Herberman, 1994). They have a unique ability to recognize and kill a variety of targets including virus infected cells and numerous types of tumour cells (Wu and Lanier, 2003) without previous sensitization (Smyth et al., 2005; Trinchieri, 1989). These cells defend against a wide range of human diseases (Whiteside and Herberman, 1994) and play an important role in tumour defence. Programmed to seek out specific targets on cancer cells that allow selective killing, NK cells are critical in the control of tumour growth and metastasis (Wu and Lanier, 2003). Their effector functions include cytotoxicity and the ability to produce a variety of cytokines (Moretta et al., 2002). Natural killer cells are activated by proinflammatory cytokines in combination with engagement of cell surface receptors (Biron et al., 1999; Smyth et al., 2005). Specific cell surface antigens mediate cell natural cytoxicity, including CD16 and CD56, which are widely used NK cell markers (Moretta et al., 2002). The potential clinical relevance of NK cells in human cancer has been demonstrated (Levy et al., 1985). Both NK cell numbers and activity appear highly responsive to psychosocial factors (Spiegel and Sephton, 2001) and subject to classical conditioning in humans

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(Buske-Kirschbaum et al., 1992). Natural killer cells can be measured in terms of their circulating numbers and their functional activity (cytotoxicity). Both vary with factors such as stress, depression, emotional states and social support (Kiecolt-Glaser et al., 1984; Landmann et al., 1984; Irwin et al., 1987; Naliboff et al., 1991; Herbert and Cohen, 1993; Esterling et al., 1994; Schedlowski et al., 1993). Indeed, NK cells respond to neuroimmune signals including the activation of endocrine stress responses (Dhabhar et al., 1995; Gan et al., 2002). The purpose of this study was to refine methods for analysing NK cell cytotoxicity and to apply the newly redefined data analysis to a patient sample.

Measurement issues

Flow cytometry with monoclonal antibodies is reliable for measuring percentages and absolute numbers of NK cells. Several methods of NK cytotoxicity assay have been established (Racz et al., 1990; Provinciali et al., 1992; Pacifici et al., 1993; Hatam et al., 1994; Friberg et al., 1996; Li et al., 1996). The 'chromium-release' method is commonly used, in which lymphocytes (effector cells) are incubated with radio-labelled tumour (target) cells at different effector-to-target (E : T) cell ratios. Radioactive chromium released from dying target cells provides a measure of lytic activity (Pross et al., 1981; Whiteside and Herberman, 1989). This permits a dose-response evaluation of killing activity over various E : T ratios.

An assay might include E : T ratios of 100 : 1, 50 : 1, 25 : 1, 12.5 : 1 and 6.25 : 1 prepared in duplicate or triplicate. After incubation and centrifugation, radioactivity is measured in the supernatant, yielding a 'cytotoxic index' of the percent of target cells killed at each ratio. A dose-response curve is generated by connecting the cytotoxic index obtained over the various E : T ratios used (Whiteside et al., 1990). Figure 1 presents the cytotoxic curve of NK cells in four breast cancer patients. Modifications to this protocol may decrease costs, increase sensitivity, or eliminate the need for radioisotopes, however, these methods still result in the generation of a cytotoxic curve (Cheknev, 2002; Friberg et al., 1996; Godoy-Ramirez et al., 2000). Suspensions of effector and target cells should be ideally prepared based on an evaluation of the percent of NK cells in each sample. An approximation of the percent of NK cells may be made with flow cytometric evaluation using the CD56 marker, and the correct E : T ratios may be plated. However, suspensions are often prepared using counts of PBMC (peripheral blood mononuclear cells) in whole blood, which actually include monocytes and other lymphoctes as well as NK cells. If so, E : T ratios may be mathematically adjusted after the assay for actual counts of NK cells (for example, obtained from flow cytometry) prior to calculation of the cytolytic curve (Friberg et al., 1996). This may be done by adjusting the E : T ratios by the percent of NK cells as shown in Table 1, Columns 1–3. Parameters of the cytolytic curve may be calculated using the adjusted E : T ratios. Other concerns arise because results are commonly presented in various formats which are difficult to compare. Even the use of lytic units, an attempt at standardization of results, varies among studies; a lytic unit (LU) is defined as the estimated number of effector cells required to kill a specified quantity of target cells. For example, LU 20 indicates the number of effector cells required to kill 20% of target cells in a suspension of standard concentration. While authors often present LU 20, some earlier researchers have used LU 33 or LU 50 (Cerottini et al., 1974; Clark et al., 1976). In an attempt to avoid the problems of lytic units, some simply present the cytotoxic index. This is also problematic because the relationship between an independent variable and NK cell activity may be significant at high E : T ratios but not at low ratios, or visa versa (Baron et al., 1990; Friberg et al., 1996). Data from our laboratory show that the effects of recent radiation or chemotherapy on NK cytoxicity may be significant at mid-range, but not high or low high E : T ratios. On one hand, the use of multiple E : T ratios provides important information, allowing for observation of findings, which might otherwise be missed. On the other hand, it also creates a data analytic problem which is the major focus of this paper: how to express NK cell activity in a manner which best reflects the quantitative function of these cells across an entire range of E : T ratios.

Materials and methods

Methods

Statistical modelling can be used to develop a mathematical approach for measuring and analysing NK cytotoxicity response curves using methods sensitive to the sources of individual differences, as well as sensitive to changes within individuals over time, or in response to different interventions. In Figure 1 response curves from four individual breast cancer patients were presented, selected to illustrate the heterogeneity of

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response curves that must be dealt with in a clinical population.

Explanatory versus descriptive mathematical models
Ideally a mathematical model is based on a full understanding of the biological processes by which the data are generated, using parameters to describe individual response curves that have specific biological meaning (an *explanatory* model). Here it is known generally that each data point that influences the response curve reflects the number and potency of NK cells relative to target cells. However, there are a number of unresolved questions regarding the killing activity of NK cells:

- Is one 'hit' sufficient to lyse a target cell or are two or more necessary?
- Are all NK cells equally potent in lysing target cells or is there some distribution of potency over NK cells within each individual?
- Are there interactions among NK or among target cells that would affect the results?

All such considerations would affect the type of mathematical model and the parameters that would be needed to define an explanatory model. The upshot is that, given the current state of knowledge, we cannot yet formulate an explanatory mathematical model.

In the absence of such information, the alternative is a *descriptive* mathematical model, which attempts to capture as much of the information as possible in an observed response curve and to do so succinctly and informatively. The parameters derived from such a model for a particular individual do not necessarily have any specific biological implications, but instead serve to create an accurate image of each cytotoxicity response curve. Ideally, given the parameters, one could recreate the true curve (observed minus error of measurement). The parameters can then be used to make the necessary comparisons within and between individuals or groups of individuals. Several such approaches are currently in the research literature.

Models proposed
One approach is to select one reference value on the E : T ratio axis of Figure 1, and to compare response curves using the vertical separation between the curves at that reference point. The difficulty is that, in the absence of parallel response curves, the conclusions that are reached differ depending on which particular reference value is selected. In Figure 1, for example, for a reference value of 100, patient 63 and 18 are identical while patient 17 and 84 are quite different; for a reference value of 6.25, patient 63 and 18 are more dissimilar than are patients 17 and 84. In some cases, response curves may even cross, in which case the relative positions of the two individuals' responses will reverse from one side of the cross-point to the other. This model is clearly inadequate in that the response at one E : T ratio is not sufficient to recreate the true toxicity curve for any patient.

An even more common approach is to select one reference value on the 'percentage lysis' axis of Figure 1 – say 20% (LU20) – and to compare curves using the horizontal separation between the curves at that reference point. Lytic unit measures are fundamentally of this type. For example, one might select a reference value of 20%, and for each patient in Figure 1, locate at what value of E : T ratio that curve crosses 20%. The

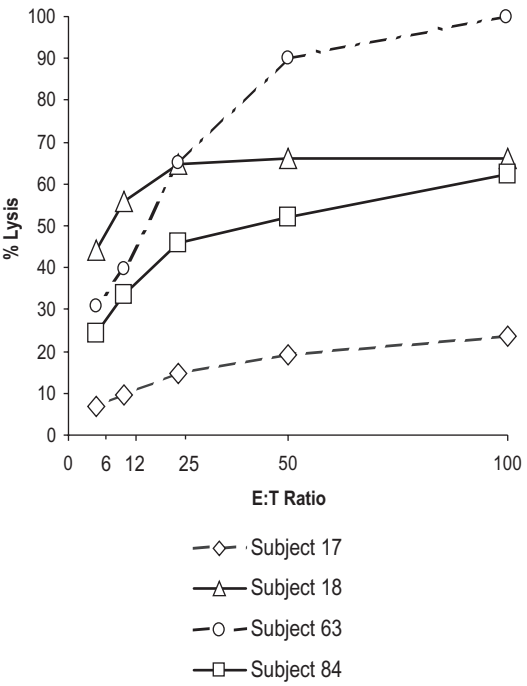

Figure 1. Cytolytic curves representing NK cell activity in four metastatic breast cancer patients, selected to show heterogeniety of response. The data shown are 'raw,' or unadjusted for the actual percent of NK cells in each blood sample.

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LU20 is based on this value. One major difficulty with this approach is indicated by the fact that for three of the four individuals in Figure 1, response curves are not observed to cross 20%, a problem that has seen little discussion in the literature. It is assumed that as the E : T ratio approaches 0, the 'percentage lysis' also approaches zero for each response curve, so for patients 63 and 18, one might extrapolate between the observed value of the response curve at E : T ratio = 6.25 and the assumed point at (0,0) to obtain an estimated crossing point. For patient 17 or any other patient whose maximal 'percentage lysis' is less than 20%, however, there is no indication that the response curve ever crosses 20%. Among individuals with highly compromised immune status, there may be many such individuals representing an important extreme of the population. Any such individual would have to be given an imputed value having no biological or descriptive meaning, or would have to be considered 'missing' in analysis. If such patients are declared 'missing', this might introduce selection bias into the sample and compromise any inferences. For this and other reasons, Pollock et al. (1991) suggest that lytic units should be used with extreme caution, if at all. In any case, even among individuals, all of whom have a non-missing value, that one value does not serve to recreate the true curve.

Perusal of response curves such as those in Figure 1 clearly indicates that one parameter does not suffice to summarize individual differences in NK cell activity across different E : T ratios among individuals. Consequently, descriptive mathematical models with at least two parameters are proposed. The easiest and most familiar such model is to assume that the response curve is adequately described by a straight line (Cheknev, 2002; Pollock et al., 1991; Pross et al., 1981):

$$L_i = A - k(100 - E : T_i),\ i = 1, 2, 3, 4, 5$$

where L_i is the observed percentage lysis at a specific E : T ratio ($E : T_i$), and i is used to index the multiple points on the individual response curve observed. Here the two parameters are *A*, which describes the % lysis at E : T ratio = 100, typically approximately the maximal observed value of % lysis, and *k*, which describes the slope of the response curve. However, as can be seen in Figure 1, response curves are typically not straight lines, and this model does not generally fit the observed response curves well (Cheknev, 2002; Pollock et al., 1991; Pross et al., 1981).

Bryant et al. (1992) have proposed instead a loglinear model (for example, Model 1, below). Two such are:

$$\text{Model 1} : L_i = A(1 - e^{-kE:T_i})$$

$$\text{Model 2} : L_i = Ae^{-k/E:T_i}$$

In both models, the two parameters are *A*, again approximating the theoretical maximal value of % lysis, and *k*, which again describes the steepness of the response curve. Model 2, our proposed model, is easier to fit using simple regression methods. Using this model:

$$\ln(L_i) = \ln(A) - k\ 1/ET_i$$

simply describes a linear model with intercept ln(*A*) and slope *k*.

Limitations of these models

Each of these models (and others including the use of cytolytic data at a single effector to target cell ratio, or a numbers of cells required to achieve a single percentage lysis value) will fit some individuals better than others. No single such model is best for all individuals at all times, as can be seen from the diversity of shapes of curves in Figure 1. In general, however, both loglinear models tend to fit most individuals quite well. Since, in order to compare different individuals, or to compare the same individual at different times, a single model must be selected; the loglinear model seems a reasonable choice.

Model 1 has been extensively recommended and used (Bryant et al., 1992). However, the computation procedures used to fit Model 1 to the observations, using these assumptions, are often done by successive approximations (iterations), where it is expected that the 'fit' of the model to the data would become closer with each iteration, eventually converging to the optimal value. When assumptions ill fit reality, solutions may converge slowly, if at all. In such cases, one must either use a questionable parameter estimate for a particular individual, or discard that individual from the sample. Either strategy introduces bias into the evaluations. For example, for patient 17 in Figure 1, one such non-linear regression fit yielded an *A* value of –124,

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well outside the possible range of 0–100. In such a case, the researcher might choose to use the obviously incorrect value of –124, treat it as an outlier, and round it to the closest value in the range, namely 0, or might choose to omit patient 17 from further analysis. Each choice is problematic. As noted by Pollack et al., (1991), biological observations cannot always be accurately expressed using mathematical descriptions. Moreover, it is possible for mathematical descriptions to introduce inaccuracies that could engender errors in the conclusions taken.

Proposed loglinear model for expressing NK cell activity

To cope with such problems, we propose to use Model 2. An example of the computation appears in Table 1.

If the loglinear model held exactly – that is, if the model perfectly fitted the data and there were no error of measurement, *k*, so estimated, would correspond exactly to that value obtained from non-linear fitting of the model. However, generally, the model will not hold exactly for every individual at every measurement point, and there will be some error of measurement. Thus the percentage of total observed variance that will be accounted for by the model (a measure of goodness of fit) will be less than 100%, but should in most cases be large (to be demonstrated below).

Validation studies

The first validation analysis was based on a sample of 502 response curve assessments for metastatic breast cancer patients for whom we had full documentation of medical treatment modality. Assessments were made on blood samples taken from all these patients weekly over several weeks. Participants were recruited and assessed yearly between 1991 and 1998 for a randomized prospective study of a psychosocial intervention. Patients had a Karnovsky rating of 70% or more, were fluent in English, and lived in the San Francisco bay area. After informed consent, patients were asked to provide blood samples on three days, approximately one week apart, between 7:00 and 10:00 am. Cytolytic activity was assessed by the chromium-release method using Ficoll-isolated cells with E : T ratios of 100 : 1, 50 : 1, 25 : 1, 12.5 : 1 and 6.25 : 1 prepared in triplicate using counts of PBMC in whole blood. NK cells were measured by flow cytometry using monoclonal antibodies to identify cells that were positive for CD56 (NKH-1 Clone) and negative for CD3 (CD3-T1 Clone) cell-surface antigens (both antibodies were received from Coulter Cytometry, Hialeah, Florida 33010). The E : T ratios were mathematically corrected using flow cytometric indications of the percentage of NK cells in each specimen. For each of the blood samples, estimates of *A* and *k* were obtained using the computation exempli-

Table 1. Example of computation of estimators of the parameters *A* and *k*

Column 1 E : T ratio	Column 2 Percentage NK cells	Column 3 True E : T ratio (Col 1 × Col 2)/100	Column 4 1/true E : T ratio	Column 5 Percentage lysis	Column 6 Lysis fraction	Column 7 Natural log of lysis fraction
100	11.5	11.5	0.087	49.6	0.496	–0.70
50	11.5	5.75	0.174	43.0	0.430	–0.84
25	11.5	2.88	0.348	38.0	0.380	–0.97
12.5	11.5	1.44	0.696	25.9	0.259	–1.35
6.25	11.5	0.72	1.391	12.8	0.128	–2.06

Model: $\mathrm{Log}(L) = \mathrm{Log}(A) - k^*(1/E : T_{true})$

Log(L) is column 7

$(1/E : T_{true})$ is column 4

Solution:

Log(L) =	–0.631
L = exp(Log(L)) =	0.532
A or maximal % lysis =	53.2%
k or NK cytotoxicity slope =	1.025

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fied in Table 1. To allow comparison of cytolytic curves generated from raw E : T ratios (based on PBMC evaluations) with adjusted E : T ratios (based on flow cytometric percents of NK cells), we also calculated *k-raw*, the slope of the uncorrected cytolytic curve. The percentage of total variance accounted for by the model was computed. Lytic units reflecting 20% lysis (LU20) were also calculated and corrected for NK cell numbers (LU20). Mean absolute numbers of NK cells and mean LU20, if observable, for the two specimens were used.

Results

The *A* values and the percentage of total variance (a measure of goodness of fit) obtained by assessing the percentage lysis on raw versus adjusted E : T ratios are identical. Because *A* approximates maximal lysis it is unchanged by the adjustment of E : T ratios for the percent of NK cells in suspensions. However, as noted above, the value of *k* equals the value of *k-raw*, divided by %CD56. Since the percentage of CD56 cells varies from one response curve to another, the information conveyed by the two values of *k* are not the same. On this sample of 502 cytotoxicity assessments the Spearman rank correlation coefficient between *k* and *k-raw* was 0.55 which was statistically significant ($p < 0.0001$), but not strong enough to suggest that *k* and *k-raw* measure exactly the same construct.

For these 502 NK cell assessments, the median percentage of variance accounted for using the above estimation procedure was 97%, with 75% of the NK cell assessments having more than 92% of the variance accounted for. Poorest fit was obtained for curves like that of patients 17 and 18 in Figure 1, where the response curve has flat areas or some reversals of direction. When using non-linear model fitting, this type of response curve may converge slowly or not at all, or the estimates obtained may be out of reasonable bounds. However, with the current procedure, parameter estimation is no more difficult for such problematic curves and the parameter estimates always remain within reasonable bounds.

The second validation study was based on a subset of 225 response curves taken from the larger sample of 502, comprising the first set of blood draws provided by each patient. Recent medical treatment information was carefully documented prior to this baseline assessment: for each patient, it was ascertained whether in the past two months they had received radiation, chemotherapy, or no medical treatment. Four patients who received both radio- and chemotherapy were excluded from the analysis. Eleven additional patients started or ended radiotherapy and/or chemotherapy between their first and last blood samples. These patients were also excluded from the analysis. The 225 response curves included data from 13 patients who had one blood sample taken, 55 who had two and 34 who had three. For each blood sample we estimated *A*, *k*, and *k*-raw. The multiple blood samples allowed assessment of sensitivity to intra-individual differences. The three treatment groups allowed assessment of sensitivity to group differences. Based on biological expectations, we would expect that with a valid measure of NK cell activity, there would be major individual differences among patients; and NK cell activity would be suppressed by recent chemo- and radiotherapy treatment. One-way ANOVAs were conducted with treatment as the independent variable and measures of NK cell activity as the dependent variable (Table 2).

Intra-individual differences

Using only patients with 3 blood samples taken (N = 34) we computed the intra-class correlation coefficient. We found that the test-retest reliability was 75% of the total variance in *A*, 58% of the total variance in *k*, and 57% of the total variance in *k-raw*. Thus all three appear to have moderate to high test-retest reliability, but A seems more reliable than either k.

In this same analysis, we compared data from the three sequential blood draws taken from each patient, there was no significant within-subjects effect of time for *A*, *k* or *k-raw* ($p = 0.46, 0.30, 0.26$, respectively). This is in accordance with our expectations because there was no intervention conducted between any two of the three baseline blood samples, and it suggests that repeated assessments might be reasonably averaged to further improve the test-retest reliability.

Inter-individual differences

The averages for each individual were compared in a one-way ANOVA between patients who had received chemotherapy or radiotherapy within the last 2 months, with those having neither for at least 6 months (see Table 1). For *A*-values, there was a significant difference between treatment groups. There was no significant difference between groups in the values of *k* or *k*-raw.

Discussion

There are several points to be brought to the attention of researchers seeking to assess natural killer cell

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Table 2. Mean, standard deviation, and results of one-way ANOVAs testing effects of treatment on NK cell activity*

Treatment		% NK cells	A	*k*	*k-raw*	Percentage lysis at each E:T ratio				
						100:1	50:1	25:1	12.5:1	6.25:1
Chemotherapy (n = 23)	mean	17.4	0.392	1.09	7.03	40.1	34.4	28.0	20.9	14.3
	SD	10.2	0.176	0.57	2.81	16.1	15.7	13.5	10.2	7.5
Radiotherapy (n = 17)	mean	14.9	0.426	1.02	7.03	43.2	36.7	30.4	23.3	14.8
	SD	4.7	0.203	0.44	3.08	18.5	18.3	15.4	11.4	7.1
No treatment (n = 46)	mean	16.3	0.539	1.17	7.29	51.2	47.4	40.1	29.8	18.4
	SD	6.8	0.217	0.57	2.07	20.9	19.1	17.9	14.7	10.1
Significance	*F*	0.57	4.64	0.57	0.11	2.86	4.87	5.00	4.10	2.05
	p	0.568	0.012	0.568	0.892	0.063	0.010	0.009	0.020	0.135
Chemotherapy versus no treatment	Effect size	0.14	0.73	0.15	0.11	0.58	0.74	0.74	0.68	0.45
Radiotherapy versus no treatment	Effect size	0.23	0.54	0.30	0.11	0.40	0.58	0.57	0.48	0.39

* As compared to patients who received no treatment, patients who received chemotherapy or radiotherapy within the preceding two months had lower NK cell activity as measured by A, but not as measured by *k* or *k-raw*. In addition, when the percentage lysis obtained at each E:T ratio was examined, treatment effects were significant at higher E:T ratios but not at the lowest E:T ratio.

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cytotoxicity with reference to experimental outcomes. First, comparability of NK cell activity response curves between one laboratory and another is not assured. This creates no problem for within- or between-subject or group comparisons in a single study, as illustrated here, but care should be taken in comparing results from different laboratories unless standardization procedures across laboratories are assured.

Second, we noted that the trafficking of NK cells into and out of the peripheral circulation may be affected by many of the same psychosocial factors that act on NK cell function (Dhabhar et al., 1995).

Third, a response in this context is a cytotoxicity response *curve*, a relationship between observed percentage lysis and selected values of the E : T ratio as seen in Figure 1. The information in such a response curve can be reasonably well summarized using as few as two parameters, one indicating the maximal value of the curve (*A*), and one indicting the rate of directional change of the curve (*k* or *k-raw*). Reducing the information to a single parameter is unlikely to yield clear information, whether that entails assuming parallel response curves, *A* = 100% for all individuals, or focusing only on one E : T value response or only on one percentage lysis response (for example, LU20). In the present study, 19 of 225, or 8.4%, of the blood samples did not include 20% lysis in the range observed. Of those that did include 20% lysis, four blood samples predicted E : T ratios of above 100 : 1 at 20% lysis (127 : 1, 382 : 1, 1355 : 1, 12200 : 1).

The use of single parameters such as LU20 may compromise the ability to detect relationships between treatment or psychosocial variables and NK cell activity. On the other hand, use of multiple such responses per individual, such as, percentage lysis at 5 points, or LU10, LU20, etc., requires adjustment for multiple testing in order to avoid proliferation of false positive responses. However, when this adjustment is done, there is some sacrifice in power to detect within subject, between subject and between group differences. Moreover, when this is done, the specific location and source of such differences is somewhat obscured. Summarizing data obtained from the various E : T ratios may also be misleading because, as in Table 2, standard deviations of the cytolytic response may increase with E : T ratios, and power to detect inter-individual differences may also vary across E : T ratios.

When two parameters (such as *A* and *k*) are used, there may be many different ways to conceptualize the parameters (such as linear versus loglinear, *k* versus *k-raw*) and different ways to estimate them (non-linear curve fitting versus linear approaches). Such ambiguity results largely because the mathematical models used to assess these curves are not explanatory (based on knowledge of the underlying biological process that generates the curves) but descriptive (meant only to evoke a clear image of the shape and location of the response curve). No single conceptualization or model will uniformly fit all individuals at all times well, and this confounds arguments based on such statistical goodness of fit.

In view of this ambiguity, we have argued against any more mathematical complexity than is absolutely necessary to clearly evoke that image, suggesting that imposing mathematical assumptions that may or may not be satisfied in the individual response curves may result in less, rather than, more precision. This is particularly so because individual response curves, as we have shown, are very diverse. Thus any stringent mathematical assumption may be appropriate for some individuals at some times and inappropriate for others. Instead, we propose a simple estimation procedure, based only on one assumption (a loglinear response curve) that seems reasonable for the majority of response curves examined, in the sense that it accounts for most of the variance most of the time, and can be applied to all response curves (it does not depend on convergence properties). This estimation procedure produces poor fit only for curves with flattened segments or reversals of direction, where more complex procedures are likely to falter as well. Furthermore we have demonstrated that the parameter estimates so produced, even when the fit is poor, readily detect individual differences as well as inter-group differences.

Generally high values of *A* and *k* are 'healthy' responses (for example, patient 63 in Figure 1). Very preliminary results, presented here only for validation purposes, indicate strong within subject consistency for both *A* and *k* for metastatic breast cancer patients, and strong differences in *A*, but not *k*, between patients who had recently received chemo- or radiotherapy and those who had received no recent medical treatment (effect size = 0.73 for chemotherapy versus no medical treatment and effect size = 0.54 for radiotherapy versus no medical treatment).

Natural killer cell cytotoxicity is an interesting measure for use in psychosocial and biomedical research

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for a number of reasons. It is a measure of function conducted reliably *in vitro* that may be sensitive to psychosocial factors and may have clinical relevance in diseases such as cancer. Therefore having a model for best utilizing data generated from this assay, which makes full use of the data collected and represents it in a manner which provides the greatest statistical power is important. The model presented here computes maximal target cell lysis and the rate of change in lysis over the range of effector to target ratios. We hope that other investigators will evaluate the utility of this model, and that it will contribute to further elucidation of the relationships among this measure of immune function, psychosocial, and medical variables.

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