Study of the physical meaning of the binding parameters involved in effector–target conjugation using monoclonal antibodies against adhesion molecules and cholera toxin

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Received 24 August 2001; accepted 24 May 2002

Abstract

In earlier work, we established a mathematical model to characterize the binding properties of cytotoxic cells to target cells. These properties can be described by the values of the maximum effector and target conjugate frequencies, $a_{\text{max}}$ and $b_{\text{max}}$, respectively, and the dissociation constant of the conjugates formed, $K_D$ (Garcia-Peñarrubia, P., Cabrera, L., Alvarez, R., and Galvez, J., J. Immunol. Methods 155 (1992) 133). Here, we address the problem of exploring the physical meaning of these parameters and their relationships with cytotoxicity. With this purpose, conjugation between a human leukemic NK cell line (NKL) and K562 tumor cells has been studied from binding isotherms obtained from data of effector $(a)$ and target $(b)$ conjugate frequencies measured by flow cytometry analysis at different effector-to-target ratios $(R)$. The results have been compared to those obtained after target cells treatment with monoclonal antibodies recognizing adhesion molecules ICAM-1 (CD54) and LFA-3 (CD58) (which are able to block some of the receptors implicated in conjugation), as well as with cholera toxin (CTX) that can modify the state of affinity of some adhesion molecules such as LFA-1 (CD11a/CD18). The results show that: (1) blocking adhesion receptors CD54 and CD58 on the surface of target cells lead to a significant decrease of $a_{\text{max}}$ and $b_{\text{max}}$, indicating that these parameters are related to the density of expression of receptors implicated in effector–target adhesion; (2) treatment of effector cells with CTX induced an increase of $K_D$, demonstrating that this parameter is associated with the effector–target affinity of the system; and (3) parallel experiments of conjugation and cytotoxicity showed that effector–target affinity and saturability influence the cytotoxic activity of the effector population. © 2002 Elsevier Science (USA). All rights reserved.

Keywords: Effector–target conjugation; Binding parameters; NK cell conjugation; NKL conjugation

1. Introduction

Cell-to-cell interactions are of crucial importance for a number of leukocyte functions [1–3]. NK cell immuno-surveillance requires the formation of effector–target cell conjugates to construct the NK cell immune synapse [4]. Previously, we have shown that the formation of conjugates is described quantitatively by binding isotherms that are characterized by three parameters, the maximum effector and target conjugate frequencies, $(a_{\text{max}}$ and $b_{\text{max}}$, respectively) and the dissociation constant of the conjugates formed $(K_D)$ [5–9]. However, the relationship between these parameters and the physical properties of the corresponding adhesion phenomena remains to be elucidated. By following considerations analogous to those used in the study of drug or hormone/receptor binding and enzyme/substrate interactions, it was assumed that $a_{\text{max}}$ and $b_{\text{max}}$ are related to effector–target saturability and thereby to the number of adhesion receptors available on the membranes of effector and target cells [8–10]. In turn, the dissociation constant of the conjugates formed $K_D$ must be a reflect of effector–target affinity [5–9]. Hence, if $a_{\text{max}}$ and $b_{\text{max}}$
are related to the density of expression of those membrane structures involved in the formation of conjugates between effector and target cells, coating these cell populations with specific monoclonal antibodies that are able to block those adhesion receptors must lead to a modification of these parameters and, subsequently, of the cytotoxic activity of the effector–target system. To test this hypothesis, the binding parameters of NKL/K562 and fresh human NK/K562 effector–target systems and cytotoxic activity of NKL/K562 have been determined after treatment of target cells with the monoclonal antibodies anti-CD54 and anti-CD58. On the other hand, if \( K_D \) is related to effector–target avidity (which will depend on the state of affinity of membrane receptors implicated in conjugation), treatment of effector cells with cholera toxin that modifies the avidity of LFA-1 for ICAM-1 without altering neither the surface expression of LFA-1 nor its Mg\(^{2+}\) binding site [11] must modify the values of \( K_D \). This hypothesis was tested by determining the binding parameters and cytotoxic activity of cholera toxin-treated NKL cells.

The results obtained reveal that coating target cells with CD54 and CD58 produces a decrease of \( \alpha_{\text{max}} \) and \( \beta_{\text{max}} \), confirming that these parameters are related to the density of expression of receptors implicated in effector–target adhesion. Also, treatment of effector cells with CTX induced an increase of \( K_D \) (which impairs the conjugation process), indicating that this parameter is related to the effector–target avidity of the system. Finally, parallel experiments of conjugation and cytotoxicity indicated that there is a direct relationship between \( \alpha_{\text{max}}, \beta_{\text{max}}, \) and \( K_D \) and the cytotoxic activity of the effector cell population.

2. Materials and Methods

2.1. mAb, media, and reagents

Cells were cultured in RPMI 1640 (Biowhittaker, Verviers, Belgium) supplemented with 10% FCS (Seromed Biochrom, Berlin, Germany) and 2 mM L-glutamine (Seromed Biochrom); here referred to as complete medium. Methods of immunofluorescence staining were described previously [12]. A FACSort flow cytometer (Becton Dickinson) was used. Monoclonal antibodies TS2/9 (CD58), TS1/11 (anti-CD11a), and TP1/36 (anti-CD43) were donated by Dr. Sanchez-Madrid (Hospital de la Princesa, Madrid, Spain), RR1/1 (CD54) was donated by Dr. T. Springer (Harvard Medical School, Boston, USA) and were used at 2.5 \( \mu \)g/mL or as dilutions (1:5) of culture supernatants or (1/200) of ascitis for flow cytometric analysis. For antibody-blocking experiments, mAbs were used in excess of up to 10 \( \mu \)g/mL. rIL2 was provided by Hoffmann La Roche (Nutley, NJ). Cholera toxin (CTX) was from Sigma Chem. (St. Louis, MO).

2.2. Effector and target cells

NKL cells, a human leukemia NK cell line donated by M.J. Robertson (Boston, MA) [13], were used as effector cells for the majority of experiments. NKL cell line was grown in RPMI 1640 supplemented with 2 mM L-glutamine, 2% human plasma, and 10 U/ml rIL2. For some experiments, freshly purified NK cells were used as effector cells. Highly enriched populations of NK cells were purified by negative selection from heparinized blood of healthy donors aged 20–40 years, supplied by the Blood Bank of Murcia, as previously described [14]. Obtained cells were routinely >85% CD3\(^+\), CD56/CD16\(^+\), and <3% CD3\(^+\). The human erythroleukemia cell line K562 and the B cell line Daudi were used as target cells. The cell lines were maintained in complete medium. Prior to use, these cells were washed twice in medium at room temperature. Viability was assessed by trypan blue exclusion (Gibco) and was higher than 95%.

2.3. Effector–target conjugation

This process was performed as described previously [14]. Briefly, effector cells were labeled green with 400 nM calcein acetoxymethylester Ca-AM (Molecular Probes, Eugene, OR) and target cells were labeled red with 253 \( \mu \)M hydroethidine HE (Molecular Probes, Eugene, OR). Both red and green dyes can be excited at a wavelength of 488 nm from an argon laser and neither affects cell viability nor conjugate formation [15]. Next, standard procedures to facilitate effector–target conjugation were performed [7–9,16]. Equal volumes (100 \( \mu \)l) of effector and target cells with concentrations ranging between 10\(^5\) and 10\(^7\) cells/ml were added to wells of 96-well U-bottom microtiter plates, incubated at 24 °C for 20 min, and centrifuged at 50 g for 5 min. The pellets were gently resuspended 3–4 times with a micropipette and kept on ice until they were acquired in the FACSort.

2.4. Measurement of conjugates by two-color flow cytometry

A FACSort flow cytometer equipped with a 488 nm argon laser was used to measure conjugates. Ca-AM (FL1 emission) and HE (FL2 emission) stained cells were used either alone or mixed to optimize the photomultiplier voltages. FL1 and FL2 data were acquired in logarithmic mode. To reduce overlapping in the emission spectra of the dyes, an alternative to FITC/PE electronic compensation was required. Usually, FL1–FL2 ranged from 0.2 to 0.3% and FL2–FL1 from 38.0 to 40.5%. Forward vs side-scatter and FL1 vs FL2 gates were set for each experiment. The percentage of effector or target cells conjugated was determined by gating the green and dual labeled events or the red and dual labeled events, respectively. A total of 8000 events per sample were counted.
2.5. Frequency of conjugation and data analysis

The frequencies of conjugation of effector cells (\(x\)) were obtained at different values of \(R = 0.2, 0.5, 1, 2, \) and 4 by analysis of the total number of effector cells bound to target cells. In these experiments, the number of effector cells was kept constant \((N = 10^5 \text{cells/tube})\). The results were expressed as percentage of bound effector cells related to the total number of acquired effector cells. The same procedure was used to quantify the corresponding frequencies of target cells (\(\beta\)), although in this case the number of target cells was kept constant \((T = 10^5 \text{cells/tube})\). All data pairs \((x, R)\) or \((\beta, R)\) were mean values from measurements performed in two or three separate tubes or wells. Then, binding isotherms were obtained by plotting \(x \text{ vs } 1/R\) (i.e., vs. the \(T : N\) ratio), or \(\beta \text{ vs } R\) [7–9]. The determination of binding parameters from these isotherms was performed using procedures previously described [8,9,16,17]. Briefly, for effector populations the dependence of \(x\) on \(R\) can be analyzed by the equation

\[
x = \frac{x_{\text{max}}}{1 + \gamma R}
\]

while for target populations the dependence of conjugate frequencies (\(\beta\)) on \(R\) is described by the expression [8,9]

\[
\beta = \frac{\beta_{\text{max}}}{1 + \frac{R}{K}}.
\]

In Eqs. (1) and (2), \(\gamma\) and \(\delta\) are parameters related to the dissociation constant, as has been previously discussed in references [8,9]. Thus, if the experiments are performed by holding constant the number of effector cells, the value of \(K_D\) is given by

\[
K_D = N(\gamma \beta_{\text{max}} - x_{\text{max}}/2)
\]

while when the experiments are carried out at constant number of target cells we have

\[
K_D = T(\delta x_{\text{max}} - \beta_{\text{max}}/2).
\]

Eqs. (3) and (4) show that to determine \(K_D\) the values of \(x_{\text{max}}\) and \(\beta_{\text{max}}\) must be known simultaneously (note that to use these expressions, the conditions \(\gamma \beta_{\text{max}} > x_{\text{max}}/2\) and \(\delta x_{\text{max}} > \beta_{\text{max}}/2\) must be held). When only one of these parameters is known, an approximate value of \(K_D\) can be obtained with the assumption \(\beta_{\text{max}} \approx x_{\text{max}}\) and using the expression

\[
K_D = x_{\text{max}} N(\gamma - 1/2)
\]

or

\[
K_D = \beta_{\text{max}} T(\delta - 1/2).
\]

Note, however, that these approximate equations can only be applied in those effector–target systems with \(\gamma\) and \(\delta > 0.5\) (when this condition is not achieved, the values of \(\gamma\) and \(\delta\) have been used as an estimation of \(K_D\), since according to Eqs. (3) and (4) they are proportional).

2.6. Application of population distributions of multiconjugates to the correction of flow cytometry frequency data

As previously discussed [14], the existence of population distributions of conjugates raises a difficult problem when conjugate frequencies are determined by flow cytometry [18,19]. This is due to the fact that flow cytometry does not make a clear difference between \(LT_n\) and \(L_nT\) conjugates. This effect was corrected proceeding as described by Rubio et al. [14].

2.7. Simplified indices for quantitation of binding efficiency

For effector populations, there are two indices that allow us to express the overall binding capacity: (a) the binding units (BU) defined by [10]

\[
BU/10^7 \text{targets} = \frac{10^7 \ x_{\text{max}} - x_p}{\gamma N \ x_p}
\]

with \(N\) being the constant number of effector cells in the binding assays and \(x_p\) the reference binding level (normally, 20%); and (b) the value of the area under the binding isotherm (AUI) in the interval [0, \(x\)] that is given by [10]

\[
\text{AUI}(0, x) = x_{\text{max}} \left( x - \gamma \ln \frac{\sqrt{x + x}}{\gamma} \right).
\]

Values of these indices were calculated introducing into these expressions the estimates value of \(x_{\text{max}}\) and \(\gamma\) obtained from Eq. (1) by regression analysis. For target populations, the corresponding expressions are

\[
BU/10^7 \text{effectors} = \frac{10^7 \ \beta_{\text{max}} - \beta_p}{\delta T \ \beta_p}
\]

\[
\text{AUI}(0, x) = \beta_{\text{max}} \left( x - \delta \ln \frac{\delta + x}{\delta} \right).
\]

2.8. Cytotoxicity

Target cells (0.5 \(\times\) 10^6 in 100 \(\mu\)l of FCS) were labeled with 50 \(\mu\)Ci 51Cr (sodium chromate, Dupont, Wilmington, DE, specific activity 470 mCi/mg) at 37°C for 1 h and then washed three times with complete medium. Next, \(5 \times 10^3\) 51Cr-labeled targets were distributed in microtiter plates in complete medium and serial dilutions of effector cells were added to each well (each dilution was performed in triplicate) to obtain appropriate effector/target ratios. Plates were centrifuged at 50g for 5 min and incubated at 37°C for 4 h. Finally, 100 \(\mu\)l supernatant from each well is read in a gamma counter.
and the percentage of lysis is obtained according to the following equation:

\[
\%\text{lysis} = \frac{\text{cpm}_{\text{experimental}} - \text{cpm}_{\text{spontaneous}}}{\text{cpm}_{\text{maximum}} - \text{cpm}_{\text{spontaneous}}} \times 100.
\]  

(11)

In this equation \(\text{cpm}_{\text{experimental}}\) corresponds to wells containing effector and target cells, \(\text{cpm}_{\text{spontaneous}}\) to wells with target cells only, and \(\text{cpm}_{\text{maximum}}\) to wells with target cells lysed by HCl 0.1 M.

2.9. Statistical analysis

The Mann–Whitney U test was used to evaluate the statistical significance between mAb-treated cells and control experiments. For cholera toxin experiments, analyses were done using a Wilcoxon’s rank sum test. Differences were statistically significant if \(P < 0.05\). All analyses were done using SPSS version 10.0 software.

3. Results

3.1. Surface expression of adhesion molecules on effector and target cells

Before coating the cells with monoclonal antibodies against adhesion molecules, we analyzed the expression of CD2 and CD11a on NKL cells (Figs. 1A and B), as well as the expression of CD54, CD58, and CD43 molecules on K562 cells (Figs. 1C–E). Next, we study the kinetics of expression of CD54, CD58, and CD43 throughout the experimental protocol of effector–target conjugation that takes approximately 30 min at room temperature. As it can be observed from Fig. 1C–E, target cell expression of CD54, CD58, and CD43 molecules was not significantly modified during that time period. Also, we examined the effect of 30 min incubation of NKL cells with 0.250 and 0.500 \(\mu\)g/ml of cholera toxin at 37 °C on the expression of CD2 and CD11a. Figs. 1A and B show that treatment with cholera toxin did not change the expression of these molecules on NKL cells.

3.2. Parameters of conjugation after blocking adhesion receptors CD54, CD58, and CD43 on target cells

Numerous studies have demonstrated that interactions between pairs of adhesion molecules LFA-1/ICAM-1 and CD2/LFA-3 play a central role in the regulation of NK cytolysis, since they are strongly involved in the formation of the “closed chamber” that appears between effector and target membranes, i.e., the NK cell immune synapse [3,4,11,20–24]. To clarify the relationship between the binding parameters and the expression of adhesion receptors on target cells, HE-marked K562 cells were preincubated separately with an excess (between 5 and 10 \(\mu\)g/ml) of mAbs anti-CD54 (RR1/1), anti-CD58 (TS2/9), and anti-CD43 (TP1/36) for 20 min at room temperature, in the absence of azide, then washed several times, and conjugated with NKL cells for a time <30 min at room temperature. The frequencies of conjugation of effector (\(\alpha\)) and target cells (\(\beta\)) were determined by FACS analysis and corrected by applying the method proposed in [14], considering a symmetric population distribution of conjugates of 4–4 with transit constants \(k = 6\) and \(k_1 = 0.2\). From these
corrected values, the corresponding isotherms were obtained and results for CD54 and CD58 are displayed in Figs. 2A and B, which are representative of several experiments showing similar results. Parameters of conjugation (\(a_{\text{max}}, b_{\text{max}},\) and \(K_D\)) and abbreviated indices of conjugation (BU and AUI) were calculated from these isotherms by using nonlinear regression analysis in the absence (control) and presence of the indicated mAb (see Table 1). These isotherms make clear that treatment with CD54 and CD58 mAb produces a significant decrease of the maximum capacity of effector and target conjugations without inducing a slant in the shape of the curves. However, treatment of K562 with mAb anti-CD43 did not induce significant changes of the corresponding isotherm compared to the control group (Fig. 2C). The inhibitory effect of CD54 and CD58 has been analyzed in Fig. 3, which displays variations of the conjugation parameters expressed as mean values of percentages of control. Thus, coating K562 target cells with mAb CD54 decreased significantly the value of \(a_{\text{max}}\) by 32.4% (mean value of six experiments). Changes of indices BU and AUI were parallel to those of \(a_{\text{max}},\) i.e., the lower is the maximum capacity of conjugation of effector cells, the lower are BU and AUI (see Fig. 3A). Similar results were obtained when the binding parameters of target cells were determined (Figs. 2B and 4A) and so, the values of \(b_{\text{max}}\) were significantly decreased by 14% (mean value of six experiments) as well as the corresponding values of the indices BU and AUI.

In turn, coating target cells with mAb CD58 also induced a decrease of the values of \(a_{\text{max}}\) and \(b_{\text{max}},\) which were more significant than for CD54. Thus, as shown in Fig. 3B, \(a_{\text{max}}\) was reduced by 45.9% \((P < 0.001,\) mean value of six experiments) and \(b_{\text{max}}\) by 41.6% \((P < 0.001,\) mean value of three experiments, see Fig. 4B). Again, changes of indices BU and AUI were parallel to those of \(a_{\text{max}}\) and \(b_{\text{max}},\) i.e., they decreased significantly in all cases (Figs. 3B and 4B). Regarding the values of \(K_D,\) Figs. 3 and 4 show that, in general, the changes of \(\gamma, \delta,\) and therefore of \(K_D,\) were not significant for both treatments. Furthermore, these results were assessed by performing conjugation experiments between fresh purified NK cells and K562 untreated (control), and previously coated with CD54 and CD58. The effects of mAb CD54 and CD58 on the parameters of conjugation were similar to those obtained with NKL effector cells, i.e., the values of \(a_{\text{max}}\) and \(b_{\text{max}},\) which decreased significantly in all cases (Figs. 3B and 4B). According to the control group, with a significant change of \(\gamma\) and \(K_D\) values (data not shown). However, treatment of K562 cells with mAb anti-CD43 did not induce significant variations of the corresponding isotherms and parameters of conjugation neither for the NKL/K562 effector–target system as shown in Fig. 2C and Table 1 nor for freshly purified NK effector cells (data not shown). Moreover, statistical analysis of mean values of percentages of control from four experiments revealed that CD43 treatment did not modify significantly the parameters of conjugation (data not shown).

These results were also assessed in the NKL/Daudi effector–target system. Thus, Daudi target cells were also coated with mAb CD54 and CD58 before performing conjugation experiments with NKL as effector cells. The results obtained showed the same tendency as the NKL/K562 effector–target system, but the percentages of inhibition of \(a_{\text{max}}\) were higher (56.2% and 88.1% for CD54 and CD58, respectively). For \(b_{\text{max}},\) these values were
52.2% for CD54 and 53.0% for CD58. Similar results were obtained for the indices BU and AUI (data not shown). These results demonstrate that there is a close relationship between the decrease in the number of adhesion receptors CD54 and CD58 available on the membrane of target cells and the values of conjugation parameters related to saturability, $a_{\text{max}}$ and $b_{\text{max}}$.

3.3. Treatment of NKL effector cells with cholera toxin

Poggi et al. [11] have described that treatment of NK effector cell populations with cholera toxin inhibits the affinity of LFA-1 without altering its level of expression on the membrane of NK cells. We have used this property with the aim of relating changes of affinity of...
the pair LFA-1/ICAM-1 to the values \( \alpha_{\text{max}}, \beta_{\text{max}}, \) and \( K_D. \) With this purpose, NKL cells were incubated with CTX at concentrations of 0.250 and 0.500 \( \mu^g/ml \) at 37°C for 30 min. These concentrations and time of incubation were previously selected by carrying out viability assays with trypan blue of NKL cultures in the presence of 0.125, 0.250, and 0.500 \( \mu^g/ml \) (data not shown). Next, the cells were washed and assays of conjugation were carried out to determine the binding parameters as before. Fig. 5 shows a representative experiment out of three that were carried out with similar results with the binding parameters given in the corresponding caption figure. This figure shows that treatment with CTX produced a slant of the corresponding isotherm due to the increase of \( \gamma, \) and therefore of \( K_D, \) but without significant changes of the maximum binding capacity. This is illustrated in Fig. 6, which displays mean values of percentages of control of \( \alpha_{\text{max}}, \gamma, K_D, \) BU, and AUI from three experiments. As we can see, the parameter related to saturability \( \alpha_{\text{max}} \) did not vary significantly (\( \alpha_{\text{max}} \) decreased a mean of 9.3%). However, the affinity constant \( K_D \) and \( \gamma \) were increased by a mean of 99.5% and 71.0%, respectively. These results indicate that the decrease in the affinity of adhesion receptors involved in effector–target conjugation induces an increase in the parameters of conjugation that were previously related to the affinity of the effector–target system, \( \gamma \) and \( K_D [5,8,9]. \) According to these results, CTX treatment of freshly purified NK cells with 0.500 \( \mu^g/ml \) did not modify the parameter related to saturability, \( \alpha_{\text{max}}, \) but induced significant variation of \( \gamma \) and \( K_D \) (data not shown).

### 3.4. Influence of the variations of the parameters of conjugation on the cytotoxic activity of NKL cells

The final goal of any treatment intended to increase the ability of conjugation in an effector–target system is to improve the efficiency of the lytic activity against the target cell population, i.e., to kill more target cells. To gain further insights into the physical meaning of the parameters of conjugation and their relationships with the lytic reaction, experiments of cytotoxicity were carried out under the experimental conditions described in previous sections.

Thus, we first studied the effect of blocking the adhesion receptors CD54 and CD58 with the mAbs (RR1/1) and (TS2/9), respectively. Fig. 7 is representative of three experiments performed with similar results. This figure shows that treatment of K562 with these mAbs produced an important decrease of percentages of lysis,

![Fig. 5. Effect of CTX treatment of NKL cells on the NKL/K562 effector–target conjugation. Binding isotherms for effector cells plotted as \( \alpha \) vs \( 1/R. \) These curves were calculated by introducing into Eq. (1) the estimates for \( \alpha_{\text{max}} \) and \( \gamma \) obtained using nonlinear regression analysis from corrected frequencies of conjugation of effector cells (\( \alpha \)) measured by flow cytometry. Binding parameters: untreated cells (○) \( (\alpha_{\text{max}} = 34.8, \gamma = 0.6, K_D = 0.35 \times 10^6 \) cells/tube, BU = 114.8, AUI = 125.3), CTX-pretreated NKL cells (▲) \( (\alpha_{\text{max}} = 40.4, \gamma = 1.7, K_D = 4.8 \times 10^6 \) cells/tube, BU = 61.2, AUI = 108.6). See Section 2 for experimental details.

![Fig. 6. Variations of the binding parameters for effector cells in the NKL/K562 effector–target system after treatment of target cells with CTX (0.5 \( \mu^g/ml \)). Data are shown as mean values ± SEM of percentages of control from three independent experiments (*P < 0.05).

![Fig. 7. Inhibition of NKL cytotoxicity after treatment of K562 target cells with monoclonal antibodies CD54 (■) and CD58 (▲). Data are shown as mean values ± SEM of percentages of cytotoxicity.](image-url)
although this percentage was higher when cells were coated with anti-CD54 (>50%) than with anti-CD58 (approximately 40%), contrarily to what occurred with conjugation experiments where the maximum capacity of conjugation was more strongly inhibited by treatment with anti-CD58 than with anti-CD54. Finally, treatment of NKL cells with cholera toxin (Fig. 8) blocked almost completely the lytic activity of these cells against K562.

4. Discussion

In the last years, we have developed theoretical models of conjugation and cytotoxicity that explained some features of these phenomena [25,26] and most predictions of the model of effector–target conjugation were assessed experimentally [5–10,14,16] and also by computer simulation [17]. Thus, by analogy with theoretical models of interaction between enzyme/substrate and hormone/receptor [8,27–29], the parameters of conjugation \( x_{\text{max}} \) and \( \beta_{\text{max}} \) were related to \( V_{\text{max}} \) and \( B_{\text{max}} \), respectively. Therefore, \( x_{\text{max}} \) and \( \beta_{\text{max}} \) would be associated with saturability that, in turn, would be directly related to the density of expression of molecular receptors involved in cell-to-cell interaction. Following similar considerations, it was suggested that the constant \( K_D \) would be related to the avidity of the effector–target system. In this paper, we have applied an experimental methodology to assess the validity of these predictions, as well as the influence exerted by \( x_{\text{max}}, \beta_{\text{max}} \) and \( K_D \) on the cytolytic activity of effector–target systems.

To correlate the parameters \( x_{\text{max}} \) and \( \beta_{\text{max}} \) with the density of expression of adhesion receptors, we have taken advantage of mAbs recognizing target adhesion molecules ICAM-1 and LFA-3, which are directly involved in effector–target conjugation through interactions with their corresponding ligands on effector cells, LFA-1 and CD2 [20–24,30,31]. The role played by LFA-1 as adhesion receptor in T and NK cell-mediated cytotoxicity through participation in the immune synapse and signaling pathways is well established [4,32,33], as well as the implication of CD2–CD58 counterreceptor pair in innate and adaptive immune adhesion [2,3,23,24]. Furthermore, the inhibitory effect on conjugation and cytotoxicity of these mAbs has been extensively reported in different effector–target systems [20,23,24,30,31,34,35].

The results obtained in the presence of CD54 and CD58 mAbs show that the decrease in the number of receptors available on target cells for NKL and fresh NK cell effector–target conjugation is directly related to the decrease of the parameters measuring the maximum conjugation capacity of effector and target cells, \( x_{\text{max}} \) and \( \beta_{\text{max}} \), respectively, i.e., the inhibitory effect is proportional to the expression of the blocked molecule. Conversely, the dissociation constant \( K_D \) remains almost unaffected. Fc-mediated or sterically hindered interactions by mAb-treatment can be excluded, since K562-treatment with anti-CD43, which is expressed at higher intensity than CD54 and CD58, did not affect the parameters of conjugation for NKL and fresh NK cell effector cell systems. Also, we have recently described the opposite effect (a significant increase of NK/target cell conjugation) when leukemic target cells were treated with mAb anti-VLA4 [36]. These results confirm that \( x_{\text{max}} \) and \( \beta_{\text{max}} \) are associated with saturability, i.e., the density of expression of the surface molecules involved in conjugation. The BU and AUI indices which express the overall binding capacity also show significant differences in most of these experiments. This is an expected finding, since BU and AUI depend on \( x_{\text{max}} \) and \( \beta_{\text{max}} \) as well as on \( \gamma \) and \( \delta \) which, in turn, are involved in \( K_D \).

Poggi et al. [11] described that treatment of NK cells with complete choler toxin modified the affinity of the integrin LFA-1 for its ligand ICAM-1 without affecting its density of expression. This effect was not mediated by the increase of AMPe, since treatment of NK cells with pertussis toxin and forskolin, that also increase intracellular AMPe, did not modify NK cell adhesion mediated by LFA-1. They concluded that changes of LFA-1 affinity state are produced by changes of LFA-1 cytoskeletal protein associations. Here, we have adopted this approach to assess whether \( K_D \) is related to the effector–target avidity, and thus, with the affinity state of adhesion receptors. Conjugation experiments carried out in the presence of CTX showed that the parameter related to saturability, \( x_{\text{max}} \), did not change significantly, while the values of \( K_D \) (or \( \gamma \) which is proportional to this constant) underwent significant variations. This confirms that \( K_D \) is a measure of the effector–target avidity directly related to the affinity state of molecular receptors implicated in conjugation. The BU and AUI indices were also significantly decreased because, as discussed...
above, they depend on $\gamma$ (which is involved in $K_D$) and $\alpha_{\text{max}}$. In other words, these indices are a function of both saturability and affinity.

Finally, we have studied the influence of varying the parameters of conjugation on the cytotoxic activity of the NKL/K562 effector–target system. With this purpose, experiments of cytotoxicity were performed under the experimental conditions described above. Coating target cells with CD54 and CD58 mAbs systematically decreased the lytic activity of NKL effector cells with CD54, exhibiting the strongest inhibitory effect. This fact was parallel to the decrease of binding parameters $\alpha_{\text{max}}$ and $\beta_{\text{max}}$, although in this case a higher inhibition was obtained with anti-CD58. This suggests that saturability in an effector–target system is a key property in the formation of conjugates directly related to the density of expression of adhesion receptors. However, cytotoxicity is a more complex phenomenon in which additional intracellular pathways are involved. In this particular case, the lytic activity of NKL cells seems to be more dependent on the pair of adhesion molecules LFA-1-ICAM-1, despite the fact that they are less expressed on NKL and K562 than CD2-CD58. Treatment of NKL cells with CTX almost completely blocked the lytic activity against K562 in agreement with previous studies [37]. Comparison of the results of conjugation and cytotoxicity after treatment with CTX suggests that variations of effector–target avidity (determined through $K_D$ or $\gamma$) have more influence on the final result of the cytotoxic reaction than the variations of the parameters related to saturability, $\alpha_{\text{max}}$ and $\beta_{\text{max}}$. However, and since the inhibition of lysis produced by CTX treatment can be attributed to changes induced by the toxin on some second messenger pathways like the increase of $[\text{Ca}^{2+}]_i$, the inositolphosphate hydrolysis that occurs during the course of these reactions [11], or ADP ribosylation of G proteins in NK cell membranes [37], further studies should be performed before drawing this conclusion.

Acknowledgment

This work was supported by a grant from the Plan Nacional I+D FEDER, project number 1FD97-2061.

References


