Selective Activation of cAMP-dependent Protein Kinase Type I Inhibits Rat Natural Killer Cell Cytotoxicity*

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The present study examines the expression and involvement of cAMP-dependent protein kinase (PKA) isoforms in cAMP-induced inhibition of natural killer (NK) cell-mediated cytotoxicity. Rat interleukin-2-activated NK cells express the PKA α-isozymes RIIα and RIIβ, and Co and Cα contain both PKA type I and type II. Prostaglandin E2, forskolin, and cAMP analogs all inhibit NK cell lysis of major histocompatibility complex class I mismatched allogeneic lymphocytes as well as of standard tumor target cells. Specific involvement of PKA in the cAMP-induced inhibition of NK cell cytotoxicity is demonstrated by the ability of a cAMP antagonist, (R)-8-Br-adenosine 3′,5′-cyclic monophosphorothioate, to reverse the inhibitory effect of complementary cAMP agonist (S)-8-Br-adenosine 3′,5′-cyclic monophosphorothioate. Furthermore, the use of cAMP analog pairs selective for either PKA isozyme (PKA type I or PKA type II), shows a preferential involvement of the PKA type I isozyme, indicating that PKA type I is necessary and sufficient to completely abolish killer activatory signaling leading to NK cell cytotoxicity. Finally, combined treatment with phorbol ester and ionomycin maintains high levels of activating the cytolytic activity of NK cells at a site distal to the site of cAMP/PKA action.

Natural killer (NK) cells constitute a distinct lineage of lymphocytes (1) with the innate ability to specifically recognize and kill certain normal allogeneic leukocytes (2, 3), tumor cells, and virally infected cells (4, 5) by direct cell-mediated cytotoxicity. The presence of both activating and inhibitory NK cell receptors have been implicated in regulation of NK cytotoxicity. As formulated in the “missing self” hypothesis (6), a no-kill signal is induced by specific interaction of “self” MHC class I molecules with inhibitory NK receptors. Therefore, the presence of intracellular signaling pathways down-regulating or inhibiting the cytotoxic response may be important in preventing killing of normal autologous cells. In contrast to the “self” MHC class I-induced inhibition of natural killing, activating receptors have not been characterized to the same extent. However, both foreign MHC antigens on normal allogeneic cells and non-MHC antigens expressed on tumor cells have been demonstrated to interact with yet not fully characterized activating NK cell receptors (7–10). The present view is that NK cytotoxicity is a result of a fine balance between negative and positive signals induced through distinct sets of receptors inhibiting or activating the cytolytic activity (11).

The second messenger cAMP regulates a number of cellular processes (12, 13). With the exception of certain ion channels, directly regulated by cAMP (14, 15), all known effects of cAMP are mediated via activation of cAMP-dependent protein kinase (PKA). The inactive PKA holoenzyme is a tetramer consisting of two regulatory (R) and two catalytic (C) subunits (16). Two major types of PKA, PKA type I and PKA type II, display different biochemical properties due to differences in their R subunits (RI and RII, respectively) (16). Molecular cloning has revealed even greater heterogeneity of PKA subunits, and at present four regulatory (RIα, RIβ, RIIα, and RIIβ) and three catalytic subunits (Cα, Cβ, and Cγ) are known (for references, see Ref. 17). Each RI or RII subunit contain two cAMP binding sites, termed site A and site B (18), and the C subunits will be released only when both of these sites are occupied by cAMP. By using site-selective cAMP analogs, a preferential activation of either PKA type I or PKA type II is possible (19–22). Thus, by employing isozyme-selective pairs of cAMP analogs, correlation between activation of a distinct isozyme of PKA and biological responses can be obtained.

The inhibitory effect of cAMP and PKA activation on proliferation of B and T lymphoid cells is well documented (23–25). Interestingly, this regulation seems mainly to be mediated through the PKA type I isozyme. PKA type I is necessary and sufficient to confer cAMP-dependent inhibition of lymphocyte proliferation induced through the antigen receptor of T cells (26). A distinct redistribution and colocalization of PKA type I in the antigen receptors in both T cells (27) and B cells (28) is observed, supporting the notion of an isozyme-specific effect of PKA type I. Whether such direct interactions of PKA type I to receptors are limited to “mitogenic” receptors and proliferative responses or may be a general phenomenon of all antigen receptors is not known. In this context, we found it of interest to examine the effects of cAMP and a possible role of specific isozymes of PKA on NK cell cytotoxicity. This would clarify
whether PKA type I might also negatively influence acute cytotoxic and not only proliferative responses. Prostaglandin \(E_2\) and forskolin have previously been shown to exert an inhibitory effect on the cytolytic activity of NK cells toward certain tumor target cells (29–31). However, the effect of cAMP in NK allorecognition and the possibility that cAMP and specific PKA isoforms may be implicated in modulation of the killer activatory signaling pathway have not been investigated.

We have therefore addressed the effects of cAMP in both allorecognition and tumor killing and the presence and functional significance of PKA and PKA isoforms in rat IL-2-activated NK cells. The results show a restricted isozyme expression and a distinct function of the PKA type I isozyme, \(R\alpha_0C\alpha_{\infty}\), in regulating the cAMP-induced inhibition of NK cell cytotoxicity both toward allogeneic and tumor targets. Furthermore, the observation that activation of PKC and perhaps other \(Ca^2+\)-dependent events counteract the cAMP-induced inhibition of NK cell function indicates a site of action of PKA proximal to PKC.

MATERIALS AND METHODS

Chemicals—The cAMP analogs 8-AHA, 8-CPT, 8-MA, 8-Pip, N\(^{\beta}\)-BzU, N\(^{\alpha}\)-Phe, (8\(^{\beta}\)-p)-8-Br, and (8\(^{\alpha}\)-b)-8-Br (32) were dissolved in phosphate-buffered saline to stock concentrations of 0.01–0.1 m. Concentrations were determined by OD measurements using the respective absorption maxima and extinction coefficients for each cAMP analog. 8-AHA, 8-CPT, and N\(^{\beta}\)-BzU were obtained from Sigma, whereas 8-MA, 8-Pip, N\(^{\alpha}\)-Phe, (8\(^{\beta}\)-p)-8-Br, and (8\(^{\alpha}\)-b)-8-Br were obtained from BioLog Life Science Institute (Bremen, Germany). All solutions of cAMP analogs were sterile-filtered before storage and use. Stock solutions of forskolin (10 mm; Calbiochem), prostaglandin \(E_2\) (PGE\(_2\), 2.8 mm; Sigma), 12-O-tetradecanoylphorbol-13-acetate (TPA, 1 mm; Sigma), and ionomycin (1.3 mm; Sigma) were prepared in ethanol.

Animals—The rat strains (their MHC haplotypes indicated in parentheses) PVG (RT1\(^b\)), PVG1U (RT1\(^u\)), and PVG1VA1 (RT1\(^v\)) were originally obtained from Harlan Olac Ltd. (Rickmansworth, United Kingdom) and subsequently bred in Oslo. The rats were routinely screened for natural killing were due to modulation of the effector and not target cells. The results show a restricted isozyme expression and a distinct function of the PKA type I isozyme, \(R\alpha_0C\alpha_{\infty}\), in regulating the cAMP-induced inhibition of NK cell cytotoxicity both toward allogeneic and tumor targets. Furthermore, the observation that activation of PKC and perhaps other \(Ca^2+\)-dependent events counteract the cAMP-induced inhibition of NK cell function indicates a site of action of PKA proximal to PKC.

Preparation of IL-2-activated NK Cells—The isolation was performed as described previously (33). In brief, mononuclear spleen cells were obtained by density gradient centrifugation on Lymphoprep (30 min at 1,077 g, 1.077 g/ml, Nycomed Pharma, Oslo, Norway), depleted of CD3\(^+\) cells released the beads after the first few days in culture and were shown to consist of more than 98% NKRI-1\(^+\). CD3\(^+\)+CD5\(^+\)+TCR\(\alpha\beta\) NK cells as routinely assessed by flow cytometry (33).

Target Cells—Concanavalin A-activated blasts were generated as described (8). Lymphoprep-separated spleen cells were adjusted to 2 \(\times 10^6\) cells/ml and cultured for 3 days in RPMI 1640 supplemented with 5% FCS, 5% normal rat serum, 2 m M glutamine, 1 m M sodium pyruvate, 5 \(\times 10^{-5}\) m 2-mercaptoethanol, and rat recombinant IL-2 (2 U/ml; Genzyme, Cambridge, MA). As shown, similar results were obtained as when the cAMP analog was present during the whole experiment (8-CPT, both). In contrast, preincubation of target cells with 8-CPT was without effect (8-CPT, target).

To ensure that cAMP analogs to be employed in the subsequent studies or breakdown products of these analogs would not influence the \(^{51}\)Cr release from target cells, the spontaneous release of \(^{51}\)Cr from target cells in the absence of effector cells was measured in the presence of high doses of all analogs employed in the study. Spontaneous release was between 5 and 10% of total \(^{51}\)Cr incorporated, and no effect of any of the cAMP analogs on the level of spontaneous release was observed (data not shown). By trypan blue exclusion, the viability of both effector and target cells was constantly >90% and not affected by treatment with any of the cAMP analogs.

Effect of cAMP on Cytotoxicity at Different Ratios between Efferent (E) and Target (T) Cells: Concentration-dependent Effects of cAMP—Fig. 2A shows a typical titration curve of NK cytotoxicity toward allogeneic target cells with graded numbers.

Percentage cytotoxicity was calculated according to the following formula: specific \(^{51}\)Cr release = (cpm – SR)/(total incorporation – SR) \(\times 100\).

Triplicate values at each effector/target cell ratio were included in the analysis and the intra-assay variation was routinely below 5%. The specific \(^{51}\)Cr release was usually 40–60% at an effector/target ratio of 50:1, and this represents almost 100% killing of target cells, since some radioactivity is irreversibly bound and remains particulate after lysis.

Treatment of IL-2-activated NK Cells in the Cytotoxicity Assay with Chemicals—IL-2-activated effector cells were distributed in 96-well plates and treated in triplicate with different concentrations of cAMP analogs (diluted in RPMI 1640 with 10% FCS). The cAMP analogs were added 30 min (room temperature) before the target cells, and the cytotoxicity assay was performed as above. Forskolin and PGE\(_2\) were added 10 min before target cells. In experiments combining cAMP analogs with TPA or ionomycin, the phorbol ester and the calcium ionophore were added 5 min before target cells (i.e. 25 min after cAMP analogs). All concentrations refer to the final concentration of compound in wells.

RESULTS

Identification of PKA Subunits in Rat IL-2-activated NK Cells—The expression of PKA subunits in rat NK cells was assessed in cultures of highly purified IL-2-activated NK cells containing more than 98% NKR-P1\(^\beta\),CD3\(^+\) cells (33). Northern analyses demonstrated mRNAs for \(R\alpha\), \(R\alpha_0\), and \(C\alpha\) isoforms of PKA, and Western blotting revealed immunoreactive protein for \(R\alpha\), \(R\alpha_0\), and \(C\alpha\) (data not shown). The distribution between soluble and particulate PKA activity was 2:1, and ion exchange chromatography revealed characteristic peaks of phosphotransferase activity and \([\text{H}]\)cAMP binding identified as PKA type I (71%) and PKA type II (30%) as well as some free RI (data not shown).

NK Cell-mediated Killing of Normal Allogeneic Lymphoblasts Is Inhibited by cAMP—Analysis of effects of cAMP analogs and agents that elevate cAMP on NK cell lysis of normal MHC-mismatched allogeneic lymphoblasts revealed a reproducible inhibition of NK cell cytotoxicity. To examine effects of elevated endogenous cAMP levels, IL-2-activated NK cells were treated with either PGE\(_2\) (10 \(\mu\) m) or forskolin (100 \(\mu\) m) (Fig. 1A). Furthermore, for comparison, some cells were also treated with a cAMP analog (8-CPT, 300 \(\mu\) m). Fig. 1 shows that treatment with PGE\(_2\) and forskolin reduced cytotoxicity from 55 to 16% specific \(^{51}\)Cr release, whereas the effect of 8-CPT completely abolished NK-mediated cytosis (Fig. 1A). To ascertain that the observed effects of cAMP analogs were due to modulation of effector cells and not target cells, 8-CPT-pretreated effector cells were washed to remove the analog prior to addition to the target cells (Fig. 1B; 8-CPT, effector). As shown, similar results were obtained as when the cAMP analog was present during the whole experiment (8-CPT, both). In contrast, preincubation of target cells with 8-CPT was without effect (8-CPT, target).

This showed that the observed inhibitory effects of cAMP on natural killing were due to modulation of the effector and not the target cells.

To ensure that cAMP analogs to be employed in the subsequent studies or breakdown products of these analogs would not influence the \(^{51}\)Cr release from target cells, the spontaneous release of \(^{51}\)Cr from target cells in the absence of effector cells was measured in the presence of high doses of all analogs employed in the study. Spontaneous release was between 5 and 10% of total \(^{51}\)Cr incorporated, and no effect of any of the cAMP analogs on the level of spontaneous release was observed (data not shown). By trypan blue exclusion, the viability of both effector and target cells was constantly >90% and not affected by treatment with any of the cAMP analogs.

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The inhibitory effect of cAMP on NK-mediated cytotoxicity is specific and operates through PKA—To further investigate the involvement of PKA in cAMP-mediated inhibition of NK cell cytotoxicity, we used a cAMP antagonist (S\(_p\)-8-Br) to reverse cAMP-dependent activation of PKA. IL-2-activated NK cells were treated with increasing concentrations (0–1000 \(\mu\)M) of the cAMP agonist (S\(_p\)-8-Br) alone or pretreated with increasing concentrations of the complementary antagonist (R\(_p\)-8-Br) (0–1000 \(\mu\)M) before the addition of a fixed concentration (1000 \(\mu\)M) of (S\(_p\)-8-Br), and their abilities to lyse allogeneic lymphoblasts were measured (Fig. 3). As expected, a concentration-dependent reduction of all activity was observed with the cAMP agonist (S\(_p\)-8-Br) with an IC\(_{50}\) of \(-750 \mu\)M (additional data not shown). The inhibitory effect of (S\(_p\)-8-Br) was almost completely reversed by pretreatment of the NK cells with the cAMP antagonist (R\(_p\)-8-Br) (Fig. 3). The observation that the cAMP-mediated inhibition of NK cell cytotoxicity could be blocked by a cAMP antagonist with selectivity for PKA type I (37) clearly testifies to the fact that the effect of cAMP is mediated through PKA.

PKA Type I, but Not PKA Type II, Inhibits NK Cell Cytotoxicity toward Allogeneic Target Cells—To further investigate the possible involvement of PKA isoforms in the regulation of NK cell cytotoxicity, we used isoyme-selective cAMP analog pairs to preferentially activate PKA type I or PKA type II, as described previously (19–22). As shown in Fig. 4A, treatment of rat NK cells with a cAMP analog pair selectively activating the type I isoyme gave reproducible synergism in the inhibition of natural killing. The cAMP analog 8-MA by itself demonstrated a concentration-dependent inhibition of NK cytolysis; 8-MA...
NK-mediated Cytotoxicity Is Regulated by PKA Type I

bonds with higher affinity to the cAMP binding site B in both PKA type I and type II, but when used in higher doses it occupies both cAMP binding sites and produces a complete inhibition of NK-mediated cytotoxicity. When 8-MA was combined with a “priming” concentration of 8-Pip (2 × 10^{-4} M), a distinct shift in the apparent IC_{50} of 8-MA was observed. At this “priming” concentration, 8-Pip selects the A site only of PKA type I and thus complements 8-MA in the preferential activation of the type I isozyme but not type II. No such synergy was observed when a PKA type II-selective analog pair was used (Fig. 4B; 8-CPT with high affinity for the B site only of PKA type II combined with N^6-Bnz selecting the A site of both isozymes). Taken together, this synergistic effect with an analog pair preferentially activating PKA type I, but not with an analog pair selectively activating PKA type II, indicates that PKA type I is sufficient to mediate inhibition of NK cell cytotoxicity.

To establish that the PKA type I-synergistic effects observed in Fig. 4 (upper panels) were not unique to the particular combinations of cAMP analogs used, a panel of site-selective analogs were employed and combined in different ways. These results are summarized in Table I. The potencies of the cAMP antagonists (Table I). The degree of synergy in each combination is presented as the ratio of IC_{50}/IC_{50}.* These experiments revealed a clear synergy using type I-selective analog pairs but no such synergy using type II-selective analog pairs. Whereas the ratio IC_{50}/IC_{50} was close to unity in type II combinations, the PKA type I analog pairs gave ratios between 4 and 5. These findings provide additional strong evidence for an important role for PKA type I as a general modulator in controlling the lytic machinery in NK cells after receptor activation.

Cyclic AMP-dependent Inhibition of NK-mediated Killing of Tumor Targets Is Directed through PKA Type I—Having analyzed the effect of cAMP/PKA in MHC class I-associated natural killing of normal allogeneic lymphoblasts (Fig. 4, upper panels, and Table I), we also wanted to see if the modulatory role of PKA type I in NK cytotoxicity could be extended to encompass MHC class I-independent recognition of tumor targets. As targets we used a mouse T cell lymphoma YAC-1 (Fig. 4, middle panels) and a chemically induced mouse mastocytoma, P815 (Fig. 4, lower panels), both sensitive to IL-2-activated NK cells. Using the same technique as in the allogeneic system, IL-2-activated NK cells were treated with different combinations of site-selective analog pairs preferentially activating PKA type I (left panels) or PKA type II (right panels), and their reactivities against either YAC-1 or P-815 were examined. A concentration-dependent inhibition of cytolysis of tumor target cells by the cAMP analogs 8-MA and 8-CPT was observed with both cell lines. As illustrated, synergy was only observed when a cAMP analog pair (8-MA and 8-Pip) activating PKA type I was used, giving IC_{50}/IC_{50} ratios of 5.8 and 4 for YAC-1 and P815, respectively (Fig. 4, C and E). The analog pair 8-CPT and N^6-Bnz, complementing each other in the activation of PKA type II, gave no such synergistic effect, with IC_{50}/IC_{50} ratios of 1.03 and 1.00 (Fig. 4, D and F). Thus, the preferential activation of PKA type I appears necessary and sufficient in the regulation of NK cell-mediated killing both of...
NK-mediated Cytotoxicity Is Regulated by PKA Type I

**TABLE I**

| cAMP analog     | IC50 ± S.E. (n) | Priming analog | IC50 ± S.E. (n) | IC50/IC50 | Isozyme selected |
|-----------------|----------------|----------------|----------------|-----------|-----------------|
| 8-MA            | 2400 ± 170 (4) | 8-Pip (200 μM) | 440 ± 25 (3)   | 5.5       | I               |
| 8-AHA           | 950 ± 97 (5)   | 8-Pip (200 μM) | 220 ± 35 (3)   | 4.3       | I               |
| (N6)-cBIMPS     | 120 ± 10 (4)   | (N6)-Phe (10 μM) | 100 ± 10 (2)  | 1.2       | I               |
| N6-Phe          | 113 ± 10 (5)   | (N6)-cBIMPS (10 μM) | 94 ± 8 (3)   | 1.2       | I               |
| 8-CPT           | 50 ± 1.5 (5)   | N6-Bnz (30 μM) | 45 ± 2 (3)    | 1.1       | II              |
| N6-Bnz          | 260 ± 40 (8)   | 8-CPT (3 μM)   | 260 ± 40 (3)  | 1.0       | II              |

**a** The potency of each analog to inhibit NK cell-mediated ctotoxicity by 50% is designated IC50 (mean ± S.E.).

**b** For experiments using analog pairs that together occupy both the A and B sites of a particular isozyme, one analog (priming analog) was used at a concentration (priming concentration) that would occupy only the one CAMP binding site it has the highest affinity for. Priming concentrations for each analog were selected as approximately 10% of the IC50 value. The priming concentrations used gave no inhibitory effect alone. The priming concentration of 8-Pip was chosen on the basis of our own experiments giving synergism and previous results in other cell systems (26). As illustrated in Fig. 4, the priming concentration of 8-Pip (200μM) complemented site B-selective analogs without any inhibitory effect alone. The combination of a site B-selective analog was selected for maximal activation of PKA type II (32, 37).

**c** The apparent IC50 in combination with different priming analogs is designated IC50/IC50*.

**d** The degree of synergistic activation by each analog pair is indicated by the ratio IC50/IC50*.

The ability of different cAMP analogs and cAMP analog pairs to inhibit NK-mediated killing of allogeneic lymphoblasts

**FIG. 5.** Effect of TPA, ionomycin, and a combination of TPA and ionomycin on cAMP-mediated inhibition of NK cell cytotoxicity. Effector cells untreated or treated with TPA (10 nm), ionomycin (4 μg/ml), or a combination of TPA and ionomycin in the absence (open bars) or presence (filled bars) of 100 μM 8-CPT (30-min pretreatment) were tested for their ability to lyse allogeneic target cells (A). Similarly, the ability of TPA and ionomycin to induce lysis of syngeneic (i.e. MHC-compatible) target cells in the absence (open bars) and presence (filled bars) of 300 μM 8-CPT was tested (B). One representative experiment of three is shown.

normal allogeneic lymphocytes and of tumor cells.

**Activation of PKC by TPA and Ionomycin Reverses the Inhibitory Effect of cAMP on NK Cell Cytotoxicity**—Protein kinase C and Ca2+ have key roles in the intracellular signaling of cells, and activation of the enzyme has a mitogenic effect on B and T cells. It has also been shown that this Ca2+-/phospholipid-dependent enzyme plays an important role in activating NK cell cytotoxicity (38). We therefore examined whether the phorbol ester TPA activating PKC, a calcium ionophore (ionomycin), or a combination of these influenced the CAMP-induced inhibition of NK-mediated killing of allogeneic lymphoblasts. As shown in Fig. 5, 100 μM 8-CPT strongly inhibited NK cell-mediated lysis, and neither TPA nor ionomycin alone were able to reconstitute cytotoxicity. In contrast, the combined treatment with TPA and ionomycin almost completely reversed the 8-CPT-induced inhibition (Fig. 5A), suggesting that the direct activation of the Ca2+ /PKC signaling pathways renders the NK cell insensitive to the effect of CAMP/PKA. The combined treatment of NK cells with TPA and ionomycin also allowed killing of syngeneic target cells (Fig. 5B), and in this case, the TPA/ionomycin-induced cytolsis was insensitive to inhibition by cAMP. This indicates that combined treatment with TPA and ionomycin short-circuits both the effect of the killer inhibitory receptors, normally preventing killing of self targets, and cAMP.

**DISCUSSION**

We have addressed the presence and functional significance of CAMP-dependent protein kinase in rat NK cells. Although both PKA type I and type II isoenzymes are present in NK cells, PKA type I is necessary and sufficient for mediating CAMP-dependent inhibition of NK cytotoxicity both toward allogeneic MHC class I-mismatched leukocytes and tumor target cells. Furthermore, treatment with TPA and ionomycin reverses CAMP-mediated inhibition of NK cytosis, indicating that the Ca2+ /PKC and CAMP signaling pathways have inverse roles in regulating the cytolytic activity of NK cells and that the targets for PKA may be proximal to activation of Ca2+ /PKC.

The α isoforms RIIα, RIIβ, and Cα are ubiquitously expressed in almost all cells and tissues (for references, see Ref. 17), and these are also the primary subunits of PKA in NK cells and constitute approximately 70% PKA type I and 30% PKA type II isozyme. It could be argued that the observation of a PKA type I-specific effect is due to the high levels of PKA type I and that putative PKA type II-specific effects could be masked by the type I enzyme. However, the isozyme-specific effects exerted by both the type I and type II isoforms appear to be more dependent on specific anchoring than on the actual amounts present within the cell (22, 26, 27, 39, 40). PKA type I-mediated inhibition of receptor-induced B and T cell proliferation is accomplished by a distinct redistribution and localization of PKA type I to the antigen receptor complexes in these cells. PKA type II, on the other hand, localizes mainly to the centrosomes in lymphocytes and is therefore more likely to serve a completely different function in these cells (27, 41). Thus, PKA type I may play an important role in all lymphoid cells as a negative regulator of receptor-induced cell proliferation and function in lymphoid cells. It is interesting that PKA type I also has been found at the initiation site of phagocytosis in neutrophilic granulocytes (42), suggesting that PKA type I may be implicated in specific recognition-associated function in all leukocytes.

Characterization and cloning of inhibitory receptors for MHC class I molecules on NK cells have been reported both in rodents and in humans (43, 44). Upon ligation with the appropriate “self” MHC class I molecule, the cytotoxic response is inhibited and target cell lysis is prevented (6). Thus, the NK receptors for MHC class I molecules may have evolved to recognize certain class I motifs within the individual with the mode “recognition: no kill.” Interestingly the inhibitory function of these receptors involves tyrosine phosphorylation of the receptor and recruitment of tyrosine phosphatases (45, 46). Hence, it seems that the inhibitory receptors act by recruiting proteins that can uncouple the activation signal. Activation of NK cells may thus proceed by a release from inhibition or by
specific recognition by certain activating molecules. There is evidence for such activating receptors both in rodents (7–9) and humans (10). Furthermore, this is supported by the notion that alloreactive NK cells in the rat, employed in this study, appear to be regulated both by negative and positive signals from classical (RT1.A) and nonclassical (RT1.C) MHC class I molecules on target cells (33, 47). Recent results indicate that the heterotrimeric G proteins Gα and Gβγ may be involved in regulating alloreactivity in rat NK cells (48). It is therefore tempting to speculate that G proteins (Gq) acting on adenylyl cyclase may represent a link between receptor-mediated recognition and the intracellular cAMP pathway in NK cells. Cyclic AMP and PKA type I may also be regulated by the level of signaling through β-adrenergic and prostaglandin E2 receptors.

Other reports have focused on the role of PKA-mediated phosphorylation of the small G proteins in regulating NK activity. Proteins of the Rho family are suspected to be involved in controlling the organization of the cytoskeleton, and RhoA is thought to act on actin filaments, thereby altering cellular conformation and regulating movement of the NK cell from one target to another (49, 50). PKA-mediated phosphorylation of RhoA serves to detach RhoA from the membrane toward the cytosol. RhoA may certainly be a substrate for a tethered PKA.

In conclusion, we report that PKA type I negatively regulates NK-mediated Cytotoxicity Is Regulated by PKA Type I.