# DEFECT OF POST-TRIGGERING NATURAL KILLER CELL MEDIATED CYTOLYTIC ACTIVITY BY SYNTHETIC HIV ANALOGUE PEPTIDES

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**Abstract:** Three kinds of peptides termed ABJ917, ABJ918 and ABJ919 were synthesized and tested their capability of inhibition of Natural Killer (NK) cell mediated cytotoxicity. ABJ917, which is conserved among various retroviral transmembrane envelope proteins inhibited NK activities of the peripheral blood lymphocytes (PBLs) of both asymptomatic carriers and normal controls. ABJ918 or ABJ919 which correspond to this conserved region also inhibited NK activities. To know the mechanism of inhibition of NK cells by these synthetic peptides, conjugate formation assay and triggering assays were then performed. ABJ917 which inhibited overall NK cytotoxicity did inhibit neither NK and target tumor cell binding nor NK cell triggering by target K562 tumor cells. These results show that the inhibition of NK cytotoxic activity by synthetic HIV peptide is caused in the stage of post-triggering. Peripheral blood $CD4^+$ cell rate of the asymptomatic carriers of HIV was almost 0% when we obtained enough NK activities and these carriers were still quite healthy. Taken together, the immunosuppression of AIDS patients is thought to be caused at least partly from some defects of post-triggering lytic activity of NK cells by HIV transmembrane peptides.

## INTRODUCTION

The hallmark of AIDS is thought to be a selective depletion of CD4 positive helper/inducer lymphocytes (Fauci, 1988). But, as far as from our knowledge and experiences, asymptomatic carriers whose CD4 positive lymphocytes are almost 0% in the PBLs are still healthy with no symptoms untill the onset of opportunistic infections. After disease manifestation, NK cytolytic activities are known to be inhibited as other immunological functions (Rook *et al.*, 1983; Wong-Staal and Gallo, 1985).

Therefore the NK cells are thought to play some important roles for initiating disease manifestation of AIDS. On the other hand, the retroviral transmembrane envelope protein p15E and its synthetic analogue peptides are reported to inhibit NK cytotoxicity (Cianciolo *et al.*, 1985; Harris *et al.*, 1987). And the mechanism of this NK suppression was the

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inhibition of post-effector target binding. Here, we tried to analyze further-*i.e.* if synthetic HIV peptide inhibit NK cell triggering or not.

## Materials and Methods

Effector cells: For the NK cell source, PBLs from HIV asymptomatic carriers or normal volunteers were prepared after Ficoll Hypaque centrifugation and depletion of plastic dish adherent cells for 30 min at 37°C from heparinized blood.

Target cells: A human myelogenous leukemia cell line K562 was maintained in 5% Fetal Bovine Serum containing RPMI1640 medium and was used as a target tumor cell.

Cytolytic assay: Cytotoxicity assays were performed in a standard 4-hr $^{51}Cr$-release assay as previously described (Pross and Baines, 1981). The following equation was used to calculate cytotoxicity: % specific lysis=[(cpm test−cpm medium)/(cpm max−cpm medium)]×100. The cpm max was determined by counting an aliquot of resuspended target cells. The cpm medium was determined in wells counting targets only with no effectors added. Lytic units (LU) were calculated from cytotoxic titration curves; 1 LU was defined as the number of effector cells required to cause 20% lysis of $5\times10^3$ targets.

Binding assay: Effector PBLs were mixed with target K562 cells at a 1:1 ratio as described previously (Roder and Kiessling, 1987). The cell suspension was centrifuged at $200\times g$ for 5 min at room temperature, followed by incubation on ice for 30 min. The pellet was gently resuspended and 200 lymphocytes were counted in triplicate samples.

Triggering assay: To detect the triggering events, phospholipid methylation assay (Hirata *et al.*, 1979) was employed. PBLs ($2.5\times10^4$ cells) with or without target K562 cells ($5\times10^3$ cells) were incubated in a total volume of 200 $\mu l$ with 20 $\mu$Ci of L-[methyl-$^3$H] methionine (87 ci/nmol, 1 Ci=$3.7\times10^{-4}$ MBq, Amersham, Amersham, UK) in 1.5 m$l$ Eppen dorf tubes. After 1 hr of incubation at 37°C, the cells were washed once with cold phosphate-buffered saline (PBS) containing 5 mM L-methionine (Sigma Chemical Co., St. Louis, MO) and twice with cold PBS to stop the reaction. The lipids in the cell pellet were extracted overnight with 750 $\mu l$ of cold chloroform/methanol (v/v=2/1). After centrifugation at $27{,}000\times g$ for 15 min, the chloroform/methanol extract was transferred to borosilicate tubes (Corning Lab. Sci. Comp., Corning, New York) and evaporated to dryness in a Rotavapor RE 120 (Buchi, Toronto, Canada). Dried samples were dissolved in 100 $\mu l$ of chloroform/methanol (v/v=2/1) and aliquots (50 $\mu l$) were chromatographed by thin-layer chromatography (TLC) on 20×20 cm silica gel G plates (Analtec Inc., Newark, Denver), using a solvent system of chloroform/propionic acid/n-propyl alcohol/distilled water, 2/2/3/1 (v/v). The front migrated approximately 15 cm. After drying and staining with iodine (Sigma), phospholipid spots were scraped and the radioactivity was measured in a liquid scintilation counter (Beckman LS 7500, Beckman, Irvine. CA).

Synthetic peptides: Three kinds of peptides composed by 17 amino acids were prepared. ABJ917 is homologous to retroviral transmembrane glycoprotein p15E (correspond to gp41 for HIV). ABJ918 and ABJ919 peptides are homologous to ABJ917 but precise structures are minimally changed.

Table 1 Amino acid sequence of the synthetic peptides

| | | |
|---|---|---|
| HIV (gp41) | (ABJ917) | Leu-Gln-Ala-Arg-Ile-Leu-Ala-Val-Glu-Arg-Tyr-Leu-Lys-Asp-Gln-Gln-Leu |
| CKS17 | (ABJ918) | Leu-Gln-Asn-Arg-Arg-Gly-Leu-Asp-Leu-Leu-Phe-Leu-Lys-Glu-Gly-Gly-Leu |
| | (ABJ919) | Leu-Gln-Asn-Arg-Arg-Gly-Leu-Asp-Leu-Arg-Tyr-Leu-Lys-Asp-Gln-Gln-Leu |

Table 2 Inhibition of NK activities of a normal volunteer during incubation

| | without | +ABJ917* | +ABJ918* | +ABJ919* |
|---|---|---|---|---|
| NK activities | 1,280† | 853 | 1,067 | 1,000 |
| (% inhibition) | | (33.4) | (16.7) | (21.9) |

*Concentration of the synthetic peptides were 5 $\mu$M
† Lytic Unit

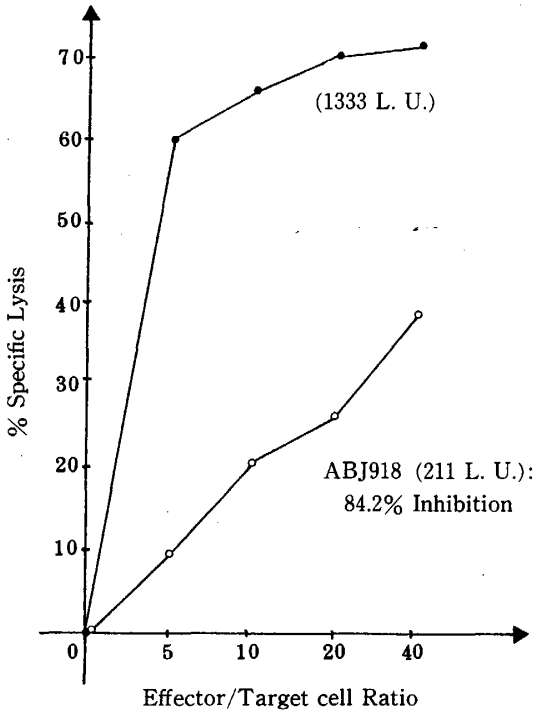


Figure 1 NK activities of the peripheral blood lymphocytes of an AIDS asymptomatic carrier before and after adding a synthetic peptide ABJ918.
Closed circle shows NK activity (1333 Lytic Unit) against $^{51}Cr$ labelled target K562 cells before adding ABJ918. Open circle shows NK activity (211 Lytic Unit) after adding the 918 peptide. 84.2% of NK activity was inhibited by 918 peptide.

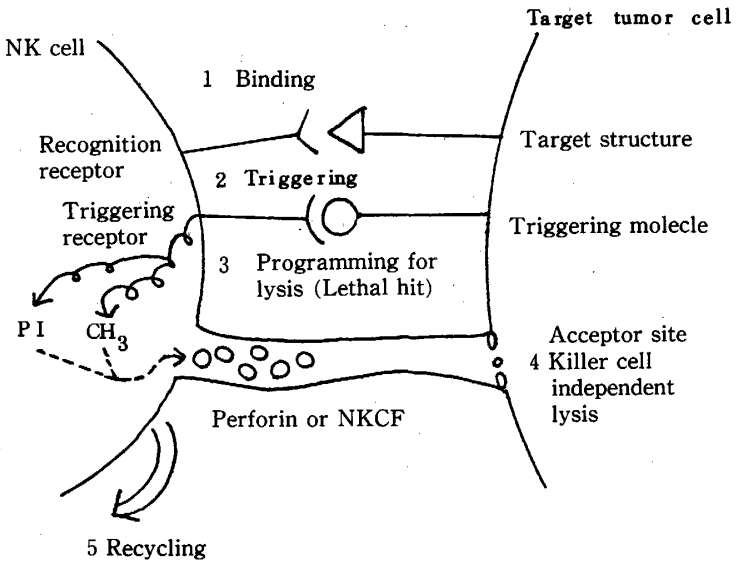


Figure 2 Schematic representation of NK cell mediated cytolytic process.
First, NK cell recognizes and binds with target tumor cells. Then, some saccharides structure (We call it triggering structure) triggers NK cell metabolic processes (Kiyohara *et al.*, 1985). Some lytic molecules are thought to be released from NK cells followed by formation of tubular structure (poly perforin) between NK and target cell and two different kinds of pores are produced on the target cell membrane. Killer cell independent lysis is the stage of target cell death accompaning DNA fragmentation (Apoptotic death). After target cell death, NK cell detach from the target and recycles to another targets.

Table 3 Percent conjugate formation of NK cells and target K562 cells

| | % conjugate formation |
|---|---|
| without peptide | 42.1±3.0 |
| with 917 peptide | 42.8±3.1 |

Concentration of the synthetic peptide 917 was 5 $\mu$M.

Table 4 The effect of a synthetic peptide 917 on the phospholipid methylation (triggering) of NK cells with K562 target cells

| Cell mixture | $^3$H-methyl incorporation into phospholipid fractions (cpm) |
|---|---|
| NK alone | 0 |
| NK+K562 | 6,871 |
| NK+K562+917 | 7,020 |

## Results and Discussion

Inhibition of NK activities of HIV asymptomatic carriers: As mentioned before, Harris *et al.* (1987) showed that NK activity of the normal volunteer was inhibited by the synthetic peptides. But, if the peptide block NK activity of the AIDS asymptomatic carrier or not has not been reported yet. So, we decided to see if our synthetic peptides inhibit NK activity of the carriers. We added 918 peptide (Table 1) to the NK assay system at the concentration

of 5 $\mu$g (Fig. 1). This 17 mer peptide inhibited NK activity of an AIDS asymptomatic carrier as much as 84%. This is surprisingly high value.

Inhibition of NK activities of a normal volunteer: Next, we tested if these peptides could inhibit NK activities of normal person instead of asymptomatic carrier. As shown in Table 2, ABJ917 as well as ABJ918 and ABJ919 inhibited normal NK activities. The difference of the magnitude of inhibition between normal persons and asymptomatic carriers may suggest some tendency or difference of sensitivity is present.

The effect of 917 peptide to NK effector target conjugate formation: NK cell mediated cytotoxicity is known to proceed via several discrete stages including: 1. effector-target cell recognition and binding, 2. triggering and activation of the NK effector cells, 3. release and binding of the lytic factor to acceptor sites on the tumor cell surface, 4. target cell death, and 5. effector cell recycling to another target cell (Fig. 2). To know which stage of these killing process is involved in peptide mediated inhibition phenomenon, we performed Roder's conjugate formation (binding) assay. As shown in Table 3, conjugate formation between NK cells and target K562 cells was not inhibited at all. Therefore, the synthetic peptide seemed to block after binding stages.

The effect of 917 peptide to NK effector cell triggering: We defined the triggering stage of NK cell cytotoxic reaction as the stage of some biochemical reactions including membrane phospholipid methylation coupled to phosphatidyl inositol turnover (Kiyohara *et al.*, 1985). So, we examined if the peptide can block phospholipid methylation (*i.e.* triggering) of NK cells. As shown in Table 4, 917 peptide did not influence NK cell triggering by K562 target tumor cells. Therefore, 917 peptide block NK cytotoxicity after triggering. Taken together, the stage of action of the synthetic peptide seemed to be after binding and after triggering. As the lytic molecules are not determined (there are some evidences that tumor necrosis factors play some roles in NK cytotoxicity and some people have reported the importance of the perforins or esterases in NK cytotoxic reaction) actual site of these synthetic peptide should be clarified in future.

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# HIVgp41の構成ペプチドおよびその類似合成ペプチドによるヒト末梢血NK活性の抑制機構

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AIDS患者末梢血NK活性が抑制されている事が知られているが，このNK活性の抑制機構を解析した。無症候性キャリアのNK活性は抑制を受けておらず，その時のキャリアの末梢血のCD4⁺細胞は0％であった。つぎに，HIVの外殻糖蛋白質gp41の一部で，レトロウイルスに保存されている17個のアミノ酸から成るペプチドと，その類似構造をしたペプチドを3種合成した。これらの合成ペプチドは，正常コントロールのNK活性も，無症候性キャリアのNK活性も共に抑制したが，キャリアに対する抑制が著しかった。正常コントロールNK活性に対する合成ペプチドの作用段階は，NK細胞と標的癌細胞の結合にも，標的細胞によるNK細胞のTriggeringにも合成ペプチドが影響を与えないことから，Triggering以後の段階であると考えられた。一般には，AIDSの発症には，HIVがCD4⁺細胞に感染し，その機能が損われる事が重要だと考えられており，多くの研究が為されているが，以上の結果より，無症候性キャリアが発症するに至るプロセスには，それ以外のNK等の免疫機能の抑制が何らかの役割を果たしていると考えられた。

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