ESRF	**Experiment title:** Structural studies on human NK cell activating receptors NKp44 and NKp46	**Experiment number**: **LS1803, LS1933**
Beamline: ID14-1 ID14-1 ID14-3	**Date of experiment**: from 09-02-01 to 12-02-01 from 23-03-2001 to 25-03-2001 from 10-06-01 to 11-06-01	**Date of report**: 10-07-01
Shifts to **BAG: 9** **BAG: 9** **BAG: 6**	**Local contact(s)**: Hassan BELRHALI Steffi ARZT Stéphanie MONACO	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Domenico Bordo*, Andrea Spallarossa* and Martino Bolognesi
Advanced Biotechnology Center
L.go R. Benzi, 10
16132 GENOVA

Introduction

Natural killer (NK) cells represent a first line of difense of the immune system against transformed and virally infected cells. NK cells lyse abnormal cell that either lack the expression or express inadequate ammounts of major histocompatibility complex (MHC) class I molecules. The ability of NK cells to discriminate between normal and neoplastic/virus-infected cells is therefore linked to the existence of triggering receptors that recognize specific non-MHC ligands on these target cells.

Some of these receptors have been recently identified and collectively named Natural Cytotoxicity Receptors (NCR). NCRs consist of glycoproteins which are expressed on the NK cellular surface and, upon interaction with the specific targets, induce cytokine production, cytotoxicity and migration. NKp44 (1) and NKp46 (2) belong to the NCR family and act as NK-specific, triggering surface proteins.

NKp44 is a 44kDa protein characterized by a single extracellular Ig V-type domain and it is selectively expressed by activated NK cells. NKp46 contains two C2-type Ig-like domains in the extracellular portion followed by a stretch of amino acids which connects the ectodomain to the transmembrane region. It represents the main receptor responsible for natural cytotoxicity and induces Ca^{++} mobilization and cytokine release. Structural studies are now in progress in order to gain insight the molecular basis of NK mediated cytolysis.

Data collection at ESRF

NKp44

A native dataset and several putative heavy atom derivatives have been collected on NKp44 crystals. Crystals belong to the $P6_22$ space group. Data analysis is currently in progress, as well as attempts to solve the structure with the molecular replacement technique. There is, however, sone indication that crystal may suffer merohedral twinning (twinning fraction close to 50%), that may hinder the structure solution until the twinning fraction is reduced to a more amenable value.

NKp46

Data collection was carried out on native crystals, to a resolution limit of 3.2Å. The space group is $C222_1$.

Due to the reduced crystal size, NKp46 structure solution should be pursued at a synchrotron site

References

(1)Vitale, M.. et al J. Exp. Med., 1998, 187, 2065-72

(2) Sivori, S. et al J. Exp. Med., 1997, 186, 1129-36

Data collection statistics on NKp44

Crystal	**native**
Space group	$P6_222$
Unit cell	a = b= 61.51, c= 201.16
Resolution	2.2 Å
Reflections	353,554
Unique reflections	20,500
Completeness	99.9
R_{merge}	7.1%
Mosaicity	0.4

Crystal	**$HgCl_2$ soak**	**$PtCl_4$ soak**	**mersalyl acid soak**
Space group	$P6_222$	$P6_222$	$P6_222$
Unit cell	a = b= 59.46, c= 196.42	a = b= 59.19, c= 197.20	a = b= 60.82, c= 198.36
Resolution	3.0 Å	3.3 Å	6.0 Å
Reflections	96,115	179,835	28,494
Unique reflections	4,673	3,623	706
Completeness	99.9%	99.9%	81.2
R_{merge}	17.3%	25.8%	84.9
Mosaicity	1.0	1.1	1.0

Crystal	**4(hydroxymercuri) benzoic acid soak**	**$HoSO_4$ soak**	**$TlCl_3$ soak**
Space group	$P6_222$	$P6_222$	$P6_222$
Unit cell	a = b= 60.23, c= 199.58	a = b= 60.79, c= 197.58	a = b= 60.14, c= 198.28
Resolution	3.0 Å	2.7 Å	3.0 Å
Reflections	101,217	199,143	170,806
Unique reflections	4,784	6,492	4,815
Completeness	98.8%	99.9%	99.9%
R_{merge}	13.2%	16.9%	19.5
Mosaicity	1.0	0.45	0.8

Crystal	**$HoSO_4$ short soak**	**$PtCl_4$ short soak**
Space group	$P6_222$	$P6_222$
Unit cell	a = b= 60.44, c= 197.80	a = b= 60.69, c= 197.56
Resolution	2.7 Å	2.4 Å
Reflections	207,647	175,296
Unique reflections	6,522	8,350
Completeness	99.9%	99.7%
R_{merge}	6.1%	6.3%
Mosaicity	0.7	0.3

Postal address: User Office, ESRF, B.P. 220, F-38043 GRENOBLE Cedex, France

Street address: 6 rue Jules Horowitz, F-38043 GRENOBLE Cedex
Telephone: +33 (0)4 7688 2552; Fax: +33 (0)4 7688 2020; e-mail: useroff@esrf.fr

Data collection statistics on NKp46

Crystal	**native**
Space group	$C222_1$
Unit cell	a = 86.24, b= 149.52, c= 59.96
Resolution	3.2 Å
Reflections	125,370
Unique reflections	6,713
Completeness	99.3%
R_{merge}	24.7%
Mosaicity	1.4

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