Serendipities of acquired immunity

Nobel Lecture
December 7, 2018

Tasuku Honjo

Kyoto University Institute for Advanced Study and Graduate School of Medicine
My family (1955)
Cassini: Earth and Saturn
The Day the Earth Smiled

“Through the brilliance of Saturn's rings, Cassini caught a glimpse of a far-away planet and its moon. At a distance of just under 900 million miles, Earth shines bright among the many stars in the sky, distinguished by its bluish tint.”

The telescopic view of Saturn fascinated me. I dreamed of becoming an astronomer.”

Saturn.
Actual photo taken on June 5, 2016
Inspired by biography of Hideyo Noguchi (1876~1928)

- Identified Syphilis spirochete as the cause of progressive paralysis
- Died in Ghana during pursuit of yellow fever pathogen
With Osamu Hayaishi
With Jacques Lucien Monod 1966
Diphtheria toxin inactivates protein synthesis factor by ADP-ribosylation

T. Honjo et al., J. Biol. Chem. (1968)
Donald Brown at Carnegie Institution in Baltimore 1971
Mystery of immune response in 1950~1970

How can animals generate antibodies specific to an almost infinite number of antigens, including artificial chemicals?
Why can animals generate specific antibodies to almost all unexperienced compounds?

- nitrobenzene-protein → Anti-N-benzene Ab
- nitrophenol-protein → Anti-N-phenol Ab
- anthracene-protein → Anti-anthracene Ab
- toluene diisocyanate-protein → Anti-toluene-DIC Ab
- oxazolone-protein → Anti-oxazolone Ab

Modified from K. Landsteiner 1919-22
Structure of antibody identified by 1970

Variable region (V)  
(Antigen-recognition site)

Constant region (C)  
(Antibody class determination)

H chain (heavy chain)  
L chain (light chain)
Philip Leder at NIH 1973
VDJ recombination generates V region repertoire during differentiation

C. Brack et al., Cell (1978)

S. Tonegawa
University of Tokyo, Dept. of Nutrition (Professor Yoshinaga Mano) 1974
Antibody memory generation by vaccine (antigen) administration

Increase in antigen binding capability (somatic hypermutation of variable region)
Somatic hypermutation (SHM) mutates V region and only good antibodies are selected.

Darwinian principle
Antibody memory generation by vaccine (antigen) administration

Increase in antigen binding capability (somatic hypermutation of variable region)

Increase in antigen processing ability (class switch of constant region)
Class switching changes the H chain constant region and antibody function

- **IgM**: Gut bacteria
- **IgA**: Parasite
- **IgG**: Virus
- **IgE**: Parasite
Class switch recombination takes place by deletion of a large DNA segment.

T. Honjo & T. Kataoka, PNAS (1978)
T. Kataoka et al., PNAS (1980)
A. Shimizu et al., Cell (1982)
The 55th Nobel Symposium
"Genetics of the Immune Response"
Saltsjobaden, Sweden, June 15 - 17, 1982

Matthias Wabl, Göran Möller (coorganizer) Leroy Hood
Discovery of AID by comparison of gene expression before and after CSR

Stimulation with CD40L, IL-4, TGF-β1

Comparison of expressed gene transcripts

Activation Induced cytidine Deaminase

Expressed in germinal center

M. Muramatsu et al., J. Biol. Chem. (1999)
Defective IgG response to antigens (Sheep Red Blood Cell) in AID deficient mice

M. Muramatsu et al., Cell (2000)
AID deficient mice fail to accumulate mutations

VH186.2 sequence (residue)

K. Kinoshita

M. Muramatsu et al., Cell (2000)
· AID deficiency in human is the cause of Hyper IgM Syndrome Type II: exactly the same phenotypes as mouse.

  P. Revy et al., Cell (2000)

· Thus, AID is the enzyme that engraves antigen memory in the antibody gene, the mechanistic basis of vaccination.
AID engraves Ab memory in the genome for effective vaccination

Repertoire formation

somatic hypermutation (SHM)

class switch recombination (CSR)

chromosomal product

Antibody memory formation

tumors

looped-out circular DNA

Natural Ab

Memory Ab
Immune surveillance against cancer

Proposed by
Sir Frank Macfarlane Burnet (1970)

However, numerous attempts to develop immunotherapy were unsuccessful.
Cancer immunotherapy by boosting accelerators has not given convincing clinical outcomes

1. Cancer vaccine

2. *In vitro* activation of T lymphocytes

3. Cytokine treatment (IFN$_\gamma$, IL-2, IL-12 etc)

This was because no immune brake molecules were known before 1995
**Brakes and accelerators control immune reactions like those in a car**

<table>
<thead>
<tr>
<th>action phase</th>
<th>drive</th>
<th>stop</th>
<th>action mode</th>
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<tbody>
<tr>
<td><strong>parking</strong></td>
<td><strong>ignition</strong></td>
<td><strong>parking brake</strong></td>
<td><strong>ON/OFF</strong></td>
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<tr>
<td>[Activation]</td>
<td>[CD28]</td>
<td>[CTLA4]</td>
<td>[Drastic]</td>
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<tr>
<td><strong>driving</strong></td>
<td><strong>accelerator</strong></td>
<td><strong>brake</strong></td>
<td><strong>~100k/h</strong></td>
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<tr>
<td>[Attack]</td>
<td>[ICOS]</td>
<td>[PD-1]</td>
<td>[Mild]</td>
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</table>
Discovery of PD-1 (programmed death-1) cDNA

Structure of cytoplasmic tail suggests PD-1 is a surface signaling molecule

Y. Ishida Y. Agata

Y. Ishida et al., EMBO J. (1992)
A long journey to understanding the function of PD-1

- **1994** PD-1 knock out (KO) on mixed background mice, no phenotype change
- **1996** PD-1 KO on C57BL/6, no phenotype change for 6M
  
  But over-response to antigen stimulation

- **1997** Nephritis and arthritis after 5M in PD-1 KO x lpr/lpr background

- **1998** Clear autoimmunity in PD-1 KO by 14M
**PD-1 is a negative regulator**

- **C57BL/6**
  - PD-1 KO (Knock Out)
  - Nephritis
  - Arthritis

- **BALB/c**
  - WT
  - PD-1 KO
  - Dilated cardiomyopathy

- **NOD**
  - NOD
  - NODxPD-1 KO
  - Diabetes

- **MRL**
  - MRL
  - MRLxPD-1 KO
  - Myocarditis

**References**

- Y. Nishimura *et al.*, Immunity (1999)
Molecular mechanism of immune inhibition by PD-1 signaling

Antigen receptor

Coreceptor

Kinase

ZAP70

Activation signal

negative signal

T. Okazaki et al., PNAS (2001)
Balance between immune surveillance and immune tolerance

Immune surveillance
Hyper immunity

PD-1 blockade

Treatment of infectious diseases and cancer
Risk of autoimmunity
Inhibition of tumorigenesis of myeloma (J558L) in PD-1−/− mice

Y. Iwai et al., PNAS (2002)
Inhibition of tumorigenesis of P815/PD-L1 by anti-PD-L1

Tumor volume (mm$^3$)

Days after inoculation

Rat IgG

a-PD-L1

P815/PD-L1 $\rightarrow$ DBA/2

N. Minato
PD-1 blockade inhibits metastasis of B16 melanoma (mouse model)

Y. Iwai et al., Int. Immunol. (2005)
PD-1 blockade by antibody against either PD-1 or PD-L1 can cure cancer.
Human anti-PD-1 antibody

Synthesized in mice containing human immunoglobulin gene by Medarex

Subclass: IgG4S228P
mutant IgG4 (S228P) stabilizes the protein and reduces ADCC
(antibody-dependent cell-mediated cytotoxicity)
KD = 2.6 nmol/L

Named Nivolumab

Approved as Investigation New Drug by FDA (USA; Aug 1, 2006)
La Jolla Institute for Allergy & Immunology
Building Opening Symposium and 5th Ishizaka Lecture
September 14, 2006

Gary Koretzky, Tasuku Honjo, Anjana Rao, Rafi Ahmed, Jim Allison, Ralph Steinman

Summary of Phase I clinical trial
296 terminal stage patients recruited
Nivolumab treatment for two years

Complete or partial response rates

18% (76 patients) of non small cell lung cancer
28% (94 patients) of melanoma
27% (33 patients) of renal cell carcinoma

S. Topalian et al., NEJM (2012)
Durable response to PD-1 blockade

“Responses were durable; 20 of 31 responses lasted 1 year or more and some even after stopping therapy”

S. Topalian et al., NEJM (2012)
### Phase II trial of anti-PD-1 antibody in patients with platinum-resistant ovarian cancer

**Dose total (n) CR PR SD PD NE RR DCR**

<table>
<thead>
<tr>
<th>Dose</th>
<th>total (n)</th>
<th>CR</th>
<th>PR</th>
<th>SD</th>
<th>PD</th>
<th>NE</th>
<th>RR</th>
<th>DCR</th>
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<td>1 mg/kg</td>
<td>10</td>
<td>0</td>
<td>1</td>
<td>4</td>
<td>4</td>
<td>1</td>
<td>1/10 (10%)</td>
<td>5/10 (50%)</td>
</tr>
<tr>
<td>3 mg/kg</td>
<td>10</td>
<td>2</td>
<td>0</td>
<td>2</td>
<td>6</td>
<td>0</td>
<td>2/10 (20%)</td>
<td>4/10 (40%)</td>
</tr>
<tr>
<td>Total</td>
<td>20</td>
<td>2</td>
<td>1</td>
<td>6</td>
<td>10</td>
<td>1</td>
<td>3/20 (15%)</td>
<td>9/20 (45%)</td>
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</table>

Tumor growth stopped in 40-50% of terminal stage patients


Oct 21, 2011-Dec 7, 2014
A responder with ovarian cancer (clear cell): Nivolumab 3mg/kg

History: 60 yr. Stage Ic with progressive disease after RSO, MMC/CPT11*3, SCH+BSO, CPT/CDDP*5, TC*2

Peritoneal dissemination ⇒ disappeared

Cancer marker CA125 (U/ml)

Durable complete responses of ovarian cancer patients to Nivolumab

Case 1

CA125 (U/ml)

No medication

> 4 years

No recurrence > 5 years

Case 2

CA125 (U/ml)

No medication

> 3.5 years

No recurrence > 4.5 years

Randomized Study on Untreated Melanoma Patients with Nivolumab and Dacarbazine (chemotherapy)

Overall survival

Hazard ratio for death, 0.42 (99.7% CI, 0.25-0.73)
P<0.001

Patients who died: median survival

Dacarbazine
Nivolumab

No./total no. mo (95% Cl)

Dacarbazine
Nivolumab

50/210 Not reached
96/208 10.8 (9.3-12.1)

No. at risk

Nivolumab
Dacarbazine

210 185 150 105 45 8 0
208 177 123 82 22 3 0

C. Robert et al., NEJM (2015)
Cancers approved for PD-1 blockade therapy

- 2014 melanoma
- 2015 lung cancer
- 2016 renal cancer
- Hodgkin's lymphoma
- head and neck cancers
- urothelial cancer
- 2017 colorectal cancer
- gastric cancer
- hepatocellular carcinoma
- Merkel cancer
- all highly mutated cancers
- 2018 cervical cancer
- primary mediastinal large B-Cell lymphoma
Paradigm shift of cancer therapy by anti-PD-1 treatment

1. Less adverse effects because normal cells are unaffected

2. Effective for a wide range of tumors (more than 1000 clinical trials)

3. Durable effects to responders after stopping treatment
Cancer cells accumulate mutations

What we learned from huge cancer genome projects

1. Cancer cells accumulate a large number of mutations to express neo-antigens that can be recognized by the immune system as non-self. This is why cancer immunotherapy is effective.

2. Too many mutations to pinpoint the dominant mutations for targeted chemotherapy.
Continuous mutations generate resistant tumor cells

- Cancer cells
- Selection mutagenesis
  - Resistant cells grow
- Drug A
- Drug B
- Lymphocytes can recognize many more mutants & attack them
Current issues in PD-1 blockade therapy

Biomarkers for responders
- High mutagenesis in tumors
- Potency of individual’s immunity

Improvement of immunotherapy
- Accessibility of killer T cells to tumor sites
- Potentiation of killer T cell function
PD-1 blockade initiates killer T cell expansion in lymph nodes

1. PD-1 blockade enhances killing within tumor which secretes chemokines

2. PD-1 blockade enhances priming and induces chemokine receptor to help migration of new killer T cell towards tumor

K. Chamoto et al., PNAS (2017)
Cancer immunotherapy by PD-1-based combination studies underway in 2017

Numbers of PD-1 blockade trials using combinations with:

1. Anti-CTLA-4 agents: 251
2. Chemotherapies: 170
3. Radiotherapies: 64
4. Anti-VEGFA agents: 43
5. Chemoradiotherapy combos: 42

Requirement of mitochondrial activation for killer T cell activation and proliferation

TCR stimulation

Mitochondria

Mitochondrial biogenesis provides cell with energy

Anti-PD-1

PD-1

Proliferation/activation of killer T cells

boost

tumor
Activation of PGC-1α/PPAR complex improves the efficacy of PD-1 blockade

K. Chamoto et al., PNAS (2017)
Bezafibrate increases killer T cell proliferation and blocks cell death

Bezafibrate + PPARα/γ

TCR stimulation → Effector killer T cells

PD-1

Exhaustion & cell death

Survival, proliferation & memory generation

Hyperimmune activity can be read in blood biochemistry of PD-1-/- mice

- Tryptophan metabolism
- Gluconeogenesis
- Malate-aspartate shuttle
- Citric acid cycle
- Urea cycle
- Galactose metabolism
- Starch and sucrose metabolism
- Fructose and mannose degradation
- Aspartate metabolism
- Ammonia recycling
- Alanine metabolism
- Glucose-alanine cycle
- Histidine metabolism
- Glutamate metabolism

PD-1−/− mice biology is very complex

Expansion of T cells

Consumption of metabolites

Gut bacterial changes

Behavioral changes

Metabolite shift

H. Nishimura et al., Immunity 1999

PD-1 selects IgA critical to microbiota regulation

IgA-coated bacteria in the gut

Less IgA-coating of bacteria in PD-1−/− mice

Bacterial dysbiosis

S. Kawamoto et al., Science (2012)
AID and PD-1 cooperate in germinal centers for high affinity IgA selection to maintain microbiome

S. Kawamoto et al., Science (2012)
Critical role of AID for controlling microbiota & whole body immune homeostasis


From Meyerholz et al., 2002
Enhanced anti-tumor immunity in AID-/- mice depends on microbiota

M. Akrami, R. Menzies, M. Miyajima, Y. Nakajima. unpublished data
Microbiome-immune system regulation

System homeostasis

Immune system

metabolites

IgA

PD1

AID

Immune tolerance
Anxiety, autoimmunity

Enhanced Anti-tumor activity

Microbiome-immune system regulation

Changes
Microbiota & Metabolites

Immune system

PD1 blockade
“We’re at the point where we’ve discovered the cancer equivalent of penicillin” says Chen. Although penicillin itself couldn’t cure all infections, it gave rise to a whole generation of antibiotics that changed medicine forever, consigning most previously fatal infections to history.
Future prospects in cancer therapy

1. Efficacy of PD-1 blockade therapy improved.

2. Many more cancers may be treated by immunotherapy.

3. Cancer may not completely disappear, but be controlled by immunotherapy. Cancer may become one of chronic diseases.
Enormous benefit by acquired immunity

20th century
Eradication of infectious diseases by vaccination and antibiotics

Pneumonia
Penicillin
Tuberculosis
Streptomycin

21st century
Cancer may be controlled by immunotherapy and its improvement including microbiome manipulation
Acquired immunity evolved in vertebrates.
Fortunate outcomes from evolution of acquired immunity

- Acquired immunity evolved in vertebrates as the defense system against pathogens. Consequently, the life span of vertebrates extended dramatically.

- Fortunately, cancer cells accumulate mutations and express neo antigens, which can also be recognized by acquired immunity.
Collaborators

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Sidonia Fagarasan
IMS, RIKEN

Fumihiko Matsuda
Kyoto University
Major outside collaborators

Antibody diversity
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M. Nussenzweig (Rockefeller Univ.)
A. Fischer (Necker Hospital)
A. Durandy (Necker Hospital)
T. Chiba (Kyoto Univ. Hospital)

Cancer immunotherapy by PD-1 blockade
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N. Minato (Kyoto Univ.)
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