Bacterial Anticancer Therapy

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Lit. Talk
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Outline

Introduction/History
  - St. Peregrine
  - Coley’s Toxins

How and why?
  - How does bacterial therapy work
  - Why would we use bacteria as therapy?

Current/future therapeutic opportunities
  - Current FDA approved therapy
  - Current clinical trials/outlook
Introduction
St. Peregrine’s Tumor

Born 1265 in Italy. Initially rebellious against religion.

Later became a priest and founded a monastery.

At the age of 60, developed malignant tumor in his foot

Tumor grew until it burst out of his foot as a lesion and became badly infected.

Amputation recommended
Introduction

St. Peregrine’s Tumor

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- Later became a priest and founded a monastery.
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Day of the surgery: The tumor appeared to be recovering

St. Peregrine lived to be 80 years old

Introduction

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“Spontaneous Regression”

likely the infection that had a curative effect

Introduction

Dr. William Coley

- Worked at New York Memorial Hospital late 1800s
- Loss of first patient in 1891 severely affected him
- Prompted search for alternatives to traditional treatment

Case: patient with sarcoma on left cheek

- repeated excisions - recurrence twice
- third attempt - partial excision
  wound left partially open
Introduction

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Case: patient with sarcoma on left cheek

- Wound gets infected
- High fever
- Tumor clears over the next 4 months

Introduction

Dr. William Coley

- Worked at New York Memorial Hospital late 1800s
- Loss of first patient in 1891 severely affected him
- Prompted search for alternatives to traditional treatment

patient still alive 7 years later

infected by:

*Streptococcus pyogenes*

Did the infection have a curative effect?

*inspired, Coley infected next 10 patients*

Introduction

Dr. William Coley

Difficulties quickly arose:

- Inconsistent infection success rate
- Dangerous/lethal infections

led to development of Coley's toxin

Introduction

Dr. William Coley

Difficulties quickly arose:

- Inconsistent infection success rate
- Dangerous/lethal infections

led to development of **Coley's toxin**

Introduction

Coley’s Toxins throughout the years

1893
Coley's toxins developed

1903
Improvement via filtration

1923
Parke-Davis sells commercially

1963
Kefauver Harris Amendment

“drug-efficacy amendment”

Introduction

Coley’s Toxins throughout the years

1963
Kefauver Harris Amendment

passed in wake of thalidomide tragedy
previous drugs are reclassified
Coley’s Toxin assigned “new drug” status
unusable outside clinical trials

“drug-efficacy amendment”

Mechanisms of Action

Local effects of bacteria


boost in M1 macrophages aids in anti-tumor response

innate immune response present at all times

LPS

M0 Macrophages

M1 Macrophages

M2 Macrophages

"attack"

"repair"
Mechanisms of Action

Local effects of bacteria

Microplasminogen  Streptokinase

Streptokinase
- bacterial enzyme from streptococcus
- forms dimer with human plasminogen
- degrades fibrin and other plasma proteins

*natively: helps bacteria avoid blot clots to aid infection*

thrombolytic medication

*in tumor conditions: may aid immune infiltration*

Mechanisms of Action

Coley’s Toxin - systemic effects

normal

T = 37 °C

Coley’s Toxin
Mechanisms of Action

Coley’s Toxin - systemic effects

Fever

thermal stress, circulation, activity

immune stimulation, trafficking, expression

Fisher, Daniel T.; Repasky, Elizabeth A.; Evans, Sharon S. Nature Reviews Immunology 2015, 15, 335–349.
Mechanisms of Action
Coley’s Toxin - systemic effects

Fever response in hypothalamus

Fisher, Daniel T.; Repasky, Elizabeth A.; Evans, Sharon S. Nature Reviews Immunology 2015, 15, 335–349.
Mechanisms of Action

Coley’s Toxin - systemic effects

**systemic fever response**

Fisher, Daniel T.; Repasky, Elizabeth A.; Evans, Sharon S. Nature Reviews Immunology 2015, 15, 335–349.
Mechanisms of Action

Coley's Toxin - systemic effects

systemic fever response

Heat-sensitive activities
- Pre-association of the TCR signalling complex
- Increased number and duration of T cell–APC interactions
- Enhanced generation of effector T cells (β2-integrin loss, IFNγ production and cytotoxic activity)

Heat-sensitive activities
- Improved L-selectin-dependent adhesion
- Increased CCL21 expression
- Enhanced intravascular density of ICAM1

Fisher, Daniel T.; Repasky, Elizabeth A.; Evans, Sharon S. Nature Reviews Immunology 2015, 15, 335–349.
Bacteria as Therapy

Why would we use bacteria therapeutically?

Genetic tune-ability

bacteria make plasmids, proteins, molecules, etc.
Bacteria as Therapy

Bacteria that manufacture proinsulin

- Harvard - initially physics, transitioned into biochemistry
- Pioneer in genomic sequencing - 1980 Nobel Prize in Chemistry
- Set out to engineer bacteria to manufacture proinsulin

**genetic recombination**

piecing together existing DNA fragments in new ways

Bacteria as Therapy

Bacteria that manufacture proinsulin

- Walter Gilbert
  - Harvard - initially physics, transitioned into biochemistry
  - Pioneer in genomic sequencing - 1980 Nobel Prize in Chemistry
  - Set out to engineer bacteria to manufacture proinsulin

**genetic recombination**

- penicillin resistance enzyme
- proinsulin gene

- penicillinase
- rat proinsulin

Bacteria as Therapy

Bacteria that manufacture proinsulin

Harvard - initially physics, transitioned into biochemistry

Pioneer in genomic sequencing - 1980 Nobel Prize in Chemistry

Set out to engineer bacteria to manufacture proinsulin

Insulin staining on culture plate, arrow indicates clone

Gilbert was narrowly beaten to this feat by Genentech who built the gene artificially

Bacteria as Therapy

Why would we use bacteria therapeutically?

- Genetic tune-ability

- Bacteria make plasmids, proteins, molecules, etc.

- We can genetically ablate toxicity
Bacteria as Therapy

Why would we use bacteria therapeutically?

- *E. coli* Nissle Strain (EcN)
  - nonpathogenic bacterial strain - discovered 1917
  - used for probiotic delivery of therapeutics

- Probiotic Mutaflor® consists of EcN
  - no toxin production
  - no pathogenic adhesion factors
  - no antibiotic resistance genes
  - no sepsis risk

Bacteria as Therapy

Why would we use bacteria therapeutically?

- Genetic tune-ability
- Natural tumor-homing activity

*bacteria will naturally migrate to necrotic tumors*
Bacteria as Therapy

Why would we use bacteria therapeutically?

- Genetic tune-ability
- Natural tumor-homing activity
- We are symbiotic with bacteria

est. ratio of human to bacterial cells is 1:1

Sender, Ron; Fuchs, Shai; Milo, Ron. PLoS Biol. 2016, 14, 8.
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How and why?
- How does bacterial therapy work
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What about bacteria in the clinic?

1. current FDA approved
Bacteria as Therapy

A current FDA-approved bacterial therapy

BCG Vaccine

Bacillus Calmette–Guérin

Developed against tuberculosis in early 1900s

Found widespread use after WWII

Adopted as standard care for bladder cancer in 1977

Bacteria as Therapy

A current FDA-approved bacterial therapy

Adopted as standard care for bladder cancer in 1977

BCG Vaccine

Bladder

Function is analogous to coley’s toxins - immune stimulation

**Bacteria as Therapy**

A current FDA-approved bacterial therapy

**Mechanism of action similar to Coley's Toxin**

Bacteria as Therapy

A current FDA-approved bacterial therapy

Adopted as standard care for bladder cancer in 1977

Function is analogous to coley’s toxins - immune stimulation

Prevents recurrence in 67% of cases of suitable candidates

In general: more effective than chemotherapy, but also more dangerous

Further investigations into colon cancer treatment are ongoing (Vaccinogen)

What about bacteria in the clinic?

1. current FDA approved
2. Salmonella
**Bacteria as Therapy**

*Salmonella for Bacterial therapy*

- Common cause of food borne illness, typhoid fever
- Intracellular pathogen/parasitic infection
- Uses secretion system to enter host cells

**Salmonella**

Salmonella typhimurium

1954

Adrenal gland tumor colonized by salmonella

**Genetic modification to reduce virulence**


Bacteria as Therapy

Salmonella for Bacterial therapy

- Deletions in *msbB* and *purl* loci - both virulence factors
- *msbB* - myristic acid moiety of lipid A
- *purl* - phosphoribosylaminoimidazole synthetase

**VNP20009**

genetically modified salmonella

*phage usage to insert DNA into Salmonella*

Bacteria as Therapy
Salmonella for Bacterial therapy

VNP20009

genetically modified salmonella

series of insertion and selection processes to arrive at VNP20009

Bacteria as Therapy

Salmonella for Bacterial therapy

- Deletions in msbB and purl loci - both virulence factors
- msbB - myristic acid moiety of lipid A
- purl - phosphoribosylaminomidazole synthetase

VNP20009

Genetically modified salmonella

Utilization in mouse xenograft model

**Bacteria as Therapy**

*Salmonella for Bacterial therapy*

- Deletions in *msbB* and *purI* loci - both virulence factors
- *msbB* - myristic acid moiety of lipid A
- *purI* - phosphoribosylaminoimidazole synthetase

**VNP20009**

genetically modified salmonella

**Utilization in mouse xenograft model**

- cytotoxicity
- immune stimulation

Deletions in *msbB* and *purl* loci - both virulence factors

- *msbB* - myristic acid moiety of lipid A
- *purl* - phosphoribosylaminomimidazole synthetase

Utilization in mouse xenograft model

Bacteria as Therapy

Salmonella for Bacterial therapy

- Deletions in *msbB* and *purl* loci - both virulence factors
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**VNP20009**

genetically modified salmonella

PBS  Dead VNP20009  Live VNP20009

Bacteria as Therapy

Clinical Trials: Salmonella

Treatment of Patients With Cancer With Genetically Modified Salmonella Typhimurium Bacteria

VNP20009
genetically modified salmonella
toxicity genetically attenuated
hypothesis - tumor homing ability in humans
strain showed tumor growth shrinkage in mice

Results:

+ No adverse effects from salmonella inoculation
- Very limited tumor colonization (3/20 patients)
- No benefit in tumor reduction observed

*terminated after phase 1 trials*

Bacteria as Therapy
Clinical Trials: Salmonella with Immunomodulators

Payload: IL-2 cytokine

increases killing activity by T-cells and NK cells

narrow therapeutic window, systemic administration difficult

Bacteria as Therapy

Clinical Trials: Salmonella with Immune Modulators

Saltikva for Metastatic Pancreatic Cancer

- localized IL-2 delivery
- improved colonization/reduced toxicity
- successful canine trials


n = 1 patient study
Bacteria as Therapy

Clinical Trials: Salmonella with Immunomodulators

Saltikva for Metastatic Pancreatic Cancer

\[ n = 1 \text{ patient study} \]

**Stage 1** | Tumor is located only in pancreas.
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**Stage 2** | Tumor involves lymph nodes and is outside pancreas (e.g. common bile duct).
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**Stage 3** | Tumor involves celiac axis or superior mesenteric artery.
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**Stage 4** | Cancer is growing in organs beyond the pancreas (e.g. liver).

Bacteria as Therapy

Clinical Trials: Salmonella with Immunomodulators

Saltikva for Metastatic Pancreatic Cancer

\[ \text{Saltikva + FOLFIRINOX administered} \]

- Folinic acid \(\text{FOL}\)
- Fluorouracil \(\text{F}\)
- Irinotecan \(\text{IRIN}\)
- Oxaliplatin \(\text{OX}\)

Stage 4 | Cancer is growing in organs beyond the pancreas (e.g. liver).

Liver

Metastasis

Bacteria as Therapy

Clinical Trials: Salmonella with Immunomodulators

Saltikva for Metastatic Pancreatic Cancer

$n = 1$ patient study

Saltikva + FOLFIRINOX administered

significant increase in NK cell levels observed

indicative of immunomodulatory effect

eventually, patient makes full recovery, healthy 24 months later

**Bacteria as Therapy**

*Clinical Trials: Salmonella with Immunomodulators*

**Saltikva for Metastatic Pancreatic Cancer**

- Localized IL-2 delivery
- Improved colonization/reduced toxicity
- Successful canine trials

**Results:**

- No adverse effects from salmonella inoculation
- Successful tumor colonization
- Full patient recovery - healthy 24 months later

*Currently in phase 2 trials*

What about bacteria in the clinic?

1. current FDA approved
2. Salmonella
3. Listeria monocytogenes
Bacteria as Therapy

Listeria monocytogenes for bacterial therapy

- Gram positive parasitic bacteria
- Cause of listeriosis - serious food poisoning condition
- Unique infection mechanism of listeria into cells

Listeria
Listeria monocytogenes

phagocytosis

Listeriolysin O

Listeriolysin O allows for lysosomal escape

Bacteria as Therapy

Clinical Trials: Listeria

Modified listeria as an HPV induced cervical cancer treatment

- Despite vaccines, HPV treatments inefficient
- Modified listeria that produce HPV antigen fusion protein

**ADXS11-001**

genetically modified Listeria monocytogenes

Cetina-Perez, Lucely et al. *Human Vaccines and Immunotherapeutics.* 2021. 17, 8, 2617–2625
Bacteria as Therapy

Clinical Trials: Listeria

*Modified listeria as an HPV induced cervical cancer treatment*

- Despite vaccines, HPV treatments inefficient
- Modified listeria that produce HPV antigen fusion protein
- T cells recognize antigen and attack HPV infected cells

**Diagram:***

- **HPV antigen**
- **Macrophage, DC**
- **T-cell**

- **MHC interaction allows T-cell to recognize HPV antigen**
- **T-cells can now kill HPV-infected cells**

Cetina-Perez, Lucely et al. *Human Vaccines and Immunotherapeutics*. 2021. 17, 8, 2617–2625
**Bacteria as Therapy**

*Clinical Trials: Listeria*

**Study of ADXS11-001 in Subjects With High Risk Locally Advanced Cervical Cancer**

- **ADXS11-001**
  - genetically modified Listeria monocytogenes

  - genetically ablated toxicity/pathogenicity
  - produces HPV fusion protein which present antigen
  - allows for T-cell mediated killing of HPV-infected cells

**Results:**

- No serious adverse side effects
- 31% of patients saw tumor size reductions
- Only 1 complete response

*currently in phase 3 trials*

Cetina-Perez, Lucely et al. *Human Vaccines and Immunotherapeutics.* 2021. 17, 8, 2617–2625
Bacteria as Therapy

Why hasn’t it been more widely adopted?

1. Toxicity

BCG vaccine accidentally contaminated with live tuberculosis strain

they were stored in the same incubator

73 infants ending up dying as a result

Lübeck disaster (1930s)
Bacteria as Therapy

Why hasn’t it been more widely adopted?

1. Toxicity

2. Efficacy

*In all live cell bacterial therapies we have discussed:*

- response rates peak at around 30%
- attenuated toxicity, but lack of true efficacy

- combination therapy
- better patient-matching
- addition of therapeutic payloads
Bacteria as Therapy

Why should we care today?

Effect of the intratumoral microbiota on spatial and cellular heterogeneity in cancer

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Bacteria as Therapy

Bacteria in the intratumoral environment

Bacterial distribution is not even

Bacterial species vary by cancer type

Bacteria as Therapy

*Bacteria in the intratumoral environment*

Bacteria change local mammalian gene expression

Bacteria as Therapy

Bacteria in the intratumoral environment

*F. nucleatum*<sup>+</sup> vs. *F. nucleatum*<sup>−</sup>

$T = 0 \text{ h}$

$T = 19 \text{ h}$

Epithelial cancer cells *F. nucleatum*

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**bacteria aid in cancer cell migration**

**bacteria induce pro-cancer expression**

Bacteria as Therapy

Why should we care today?

Effect of the intratumoral microbiota on spatial and cellular heterogeneity in cancer

in the future, we will want to consider each patient’s tumoral microbiota