6e JNI

Maladie immunoproliférative de l'intestin grêle (IPSID) associée à *Campylobacter jejuni*

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Definitions

- **IPSID** (**I**mmuno **P**roliferative **S**mall **I**ntestinal **D**isease)
  = **Alpha-heavy chain disease**
  = **Mediterranean lymphoma**
  M. Seligmann, Science 1968

- Developing countries
  - Mediterranean basin
  - Middle and Far East
  - Africa

- Characterized by
  - Small intestine **lymphoplasmacyte infiltration**
  - Secretion of a **truncated immunoglobulin alpha-heavy chain**

- WHO classification:
  - Extranodal marginal zone B-cell lymphoma
  - **MALT type** (P. Isaacson, Cancer 1983)

Efficacy of antimicrobial treatments in Gastrointestinal MALT lymphomas

Late 60's

IPSID

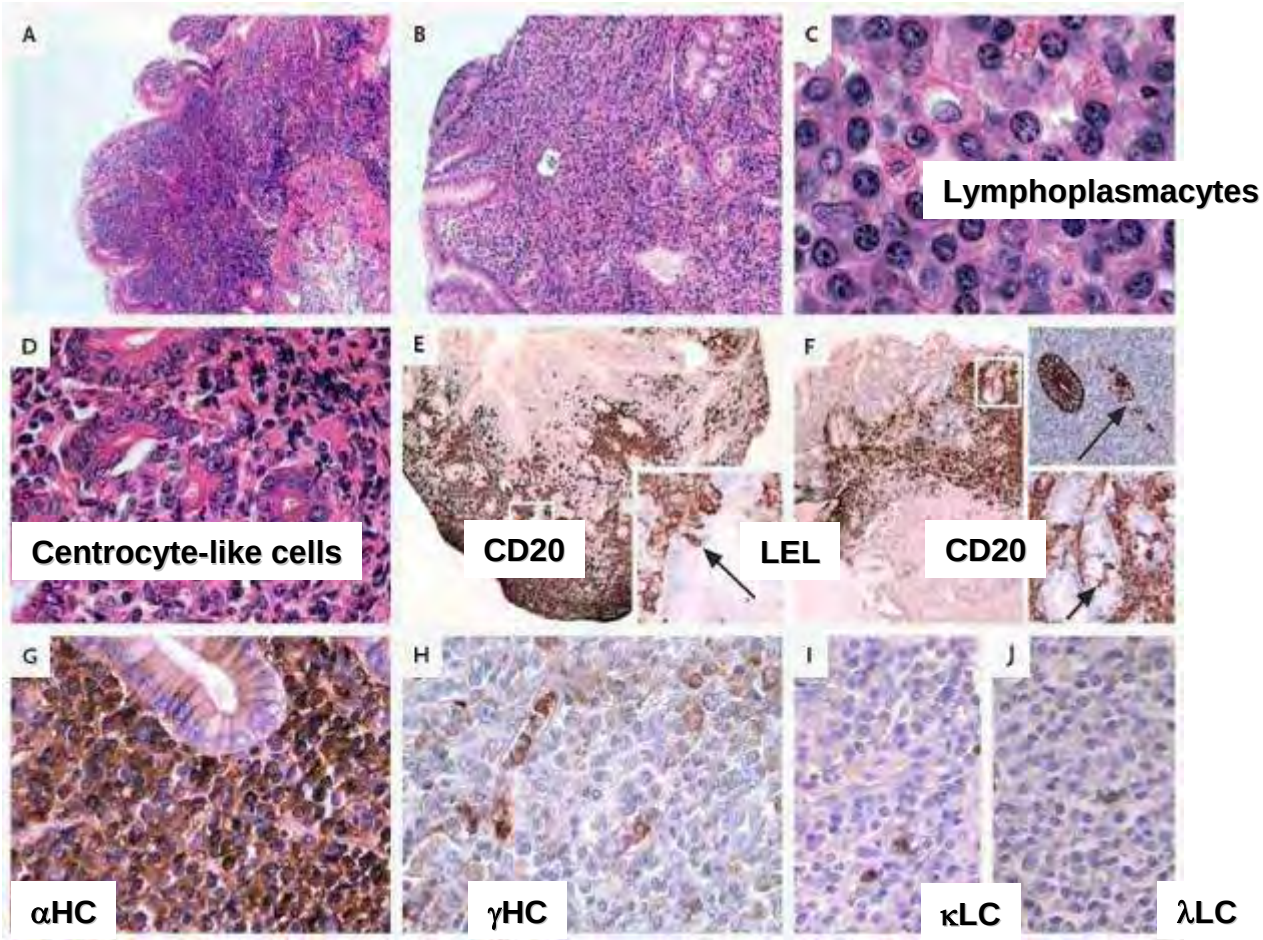
Ampicillin, Metronidazole
Tetracycline

Early 90's

Gastric lymphoma

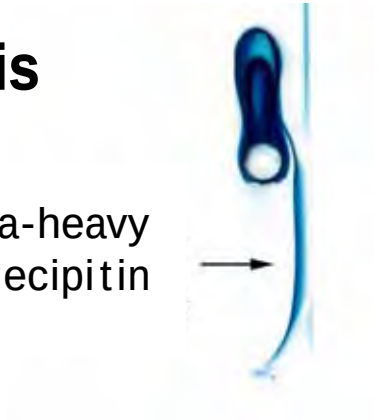
Eradication
of *H. pylori*

A case of alpha chain disease (IPSID)



A case of alpha chain disease (IPSID)

Immunoelectrophoresis



Arc of alpha-heavy chain precipitin

A case of alpha chain disease (IPSID)

Treatment

- Eradication of *H. pylori*

  Amoxicillin + Metronidazole + Clarithromycin

  Omeprazole

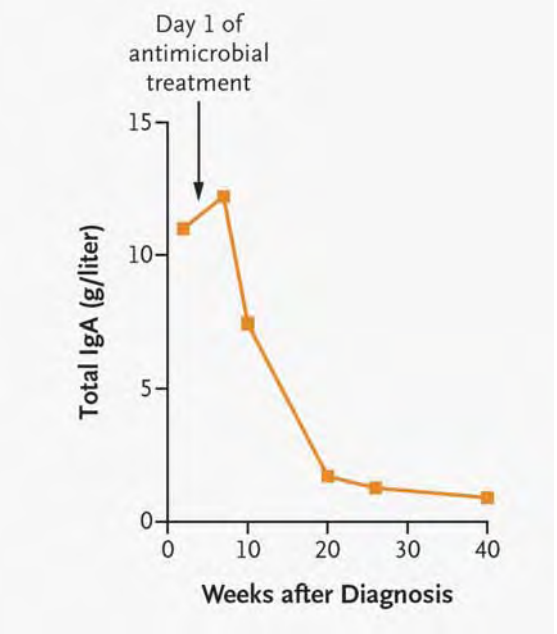
- Rationale
  - Usual efficacy of antibiotics in stage A IPSID
  - Gastric extension of the disease
  - Similarities between
    Gastric MALT lymphoma and IPSID
  - Case report of *H. pylori*-associated IPSID

A case of alpha chain disease (IPSID)

Dramatic efficacy

- Rapid regression of clinical signs
- Rapid regression of biological abnormalities

Alpha heavy chain



Total IgA

Microbiological investigations

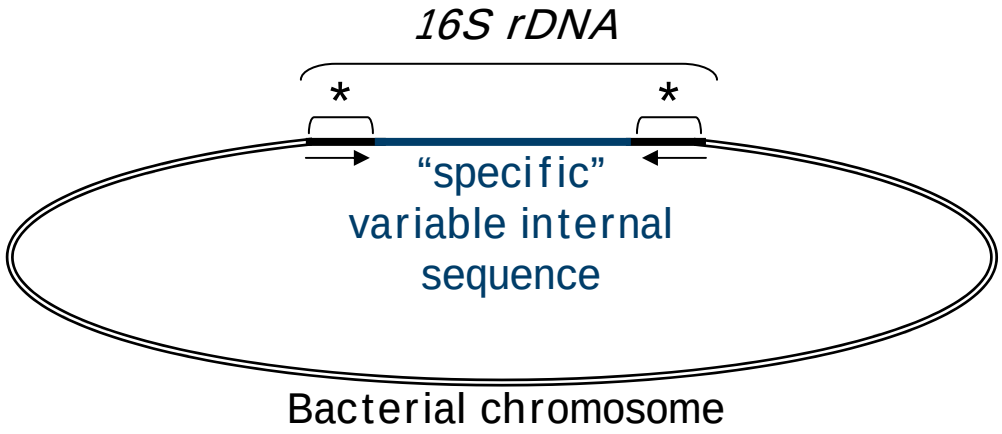
Litterature

- Microbiological investigations: unsuccessful (Harzic, 1985)
- But… potential “uncultivability”

Strategy

- No a priori
- Same as for Whipple’s disease (Relman, NEJM 1992)

↳ Identification of *Tropheryma whippelii*

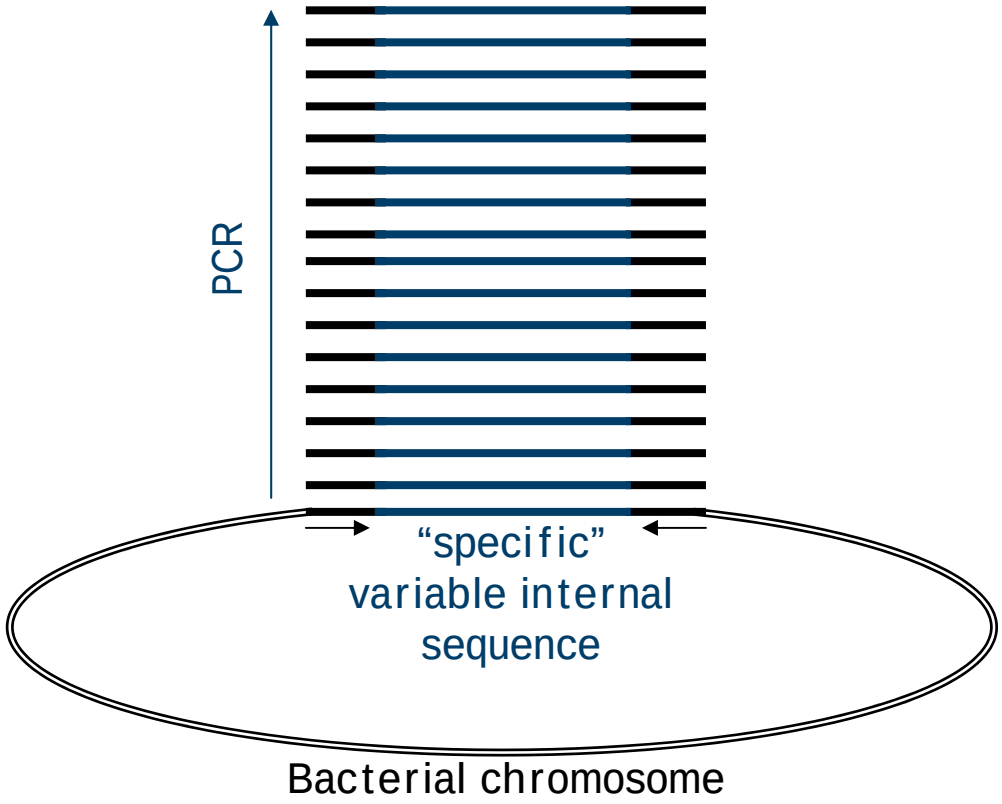


* “universal” consensus sequences

⇄ “universal” oligonucleotide pair

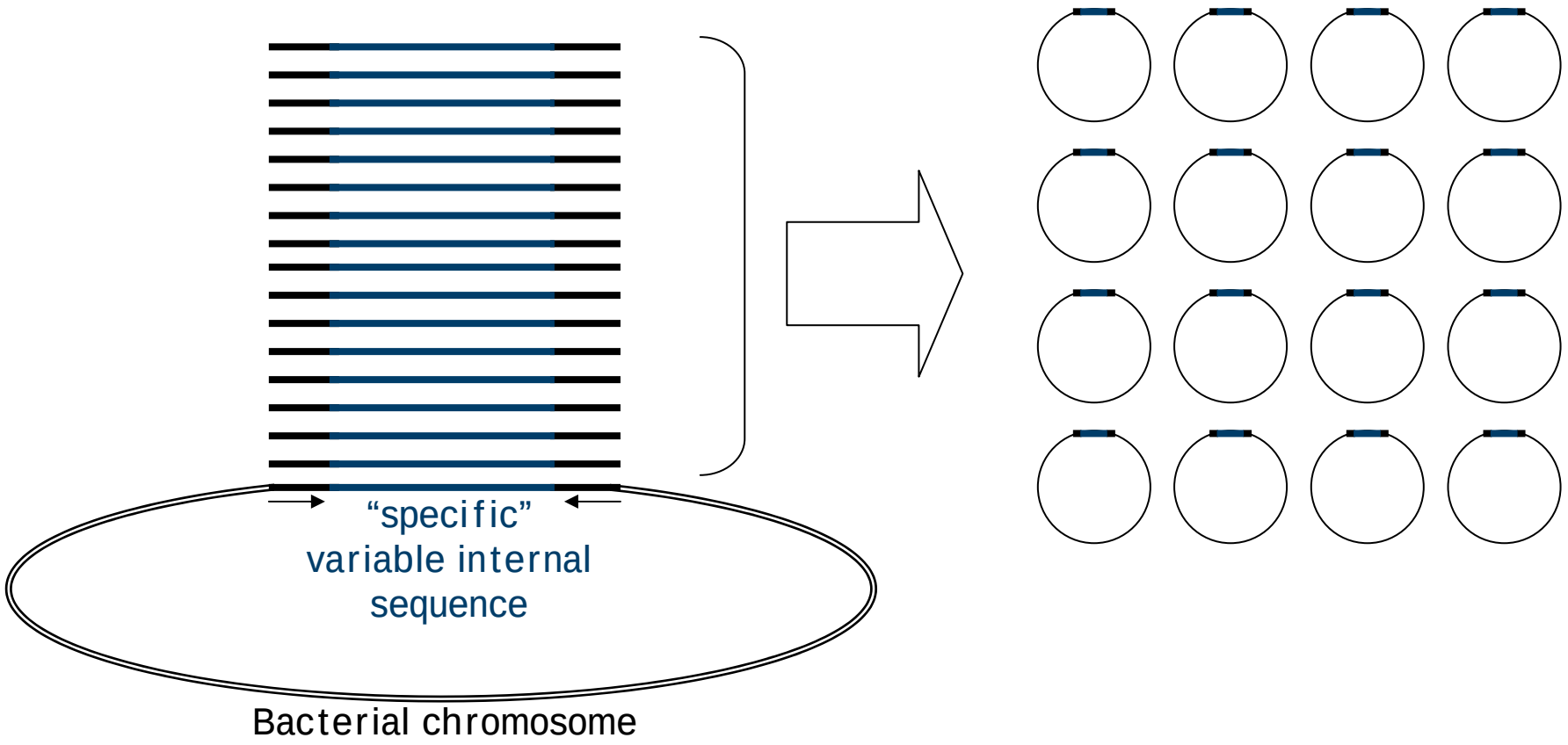
Microbiological investigations

“Universal” 16S PCR amplification



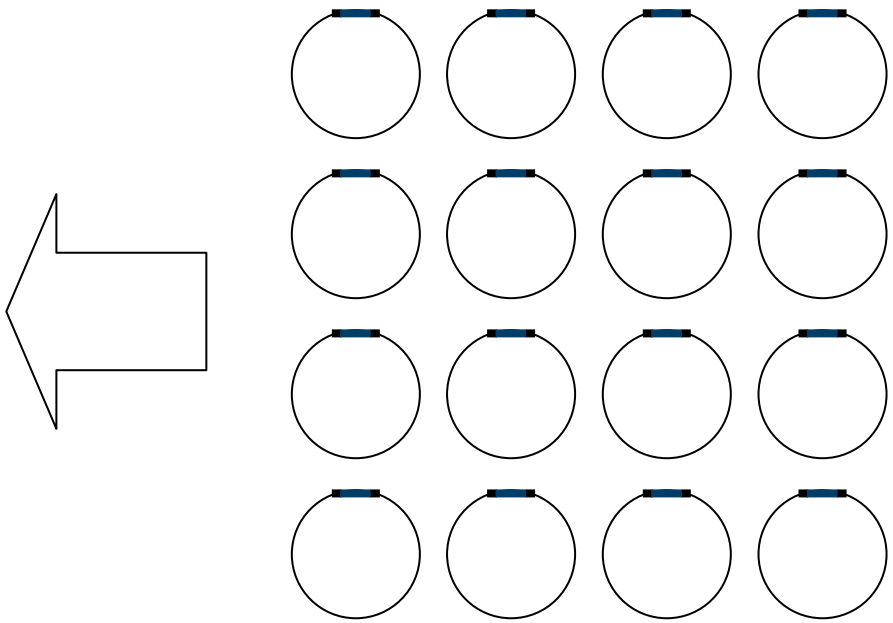
Microbiological investigations

Subcloning,Transformation, Sequencing of inserts



Microbiological investigations

Phylogenetic analysis and species identification



Microbiological investigations

Results of 16S PCR

16S PCR = positive = presence of bacterial genomes

Subcloning

Sequencing

Microbiological investigations

Insert sequences

12 independent clones

8/12 -> *Campylobacter jejuni*

4/12 ->

- 1/12 *Abiotrophia* sp.
- 1/12 *Neisseria* sp.
- 1/12 *Lactococcus* sp.
- 1/12 *Haemophilus* sp.

i.e. members of the oropharyngeal flora

Microbiological investigations

Confirmation by specific PCRs

Results of Polymerase-Chain-Reaction (PCR) Assays of Biopsy Specimens from the Index Patient with Immunoproliferative Small Intestinal Disease and Control Samples.*

Specimen	PCR Results			
	Bacterial 16S rDNA Primers	Campylobacter Primers	Helicobacter Primers	Enterobacteriaceae Primers
Controls				
Reference strain				
Campylobacter jejuni	+	+	–	–
Helicobacter pylori	+	–	+	–
Escherichia coli	+	–	–	+
Duodenum from 10 controls with diarrhea of unknown origin	ND	–	–	ND
Index patient				
Stomach and jejunum before antimicrobial treatment	+	+	–	–
Stomach and jejunum on day 8 of antimicrobial treatment	–	–	–	–
Stool before antimicrobial treatment	ND	+	–	ND

Microbiological investigations

In situ hybridization

- Objective

  Visualize *C. jejuni* within the IPSID tissue

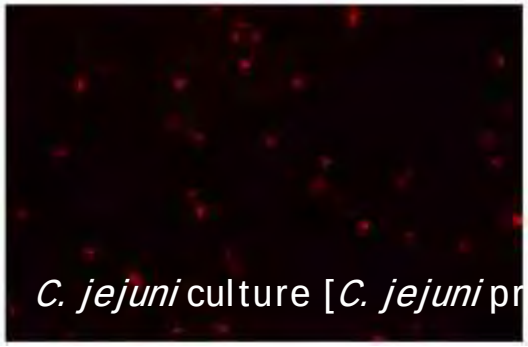
- Method

  Generation of a DNA probe (*Cj*-490)
  hybridizing specifically with *C. jejuni* 16S RNA

Microbiological investigations

In situ hybridization

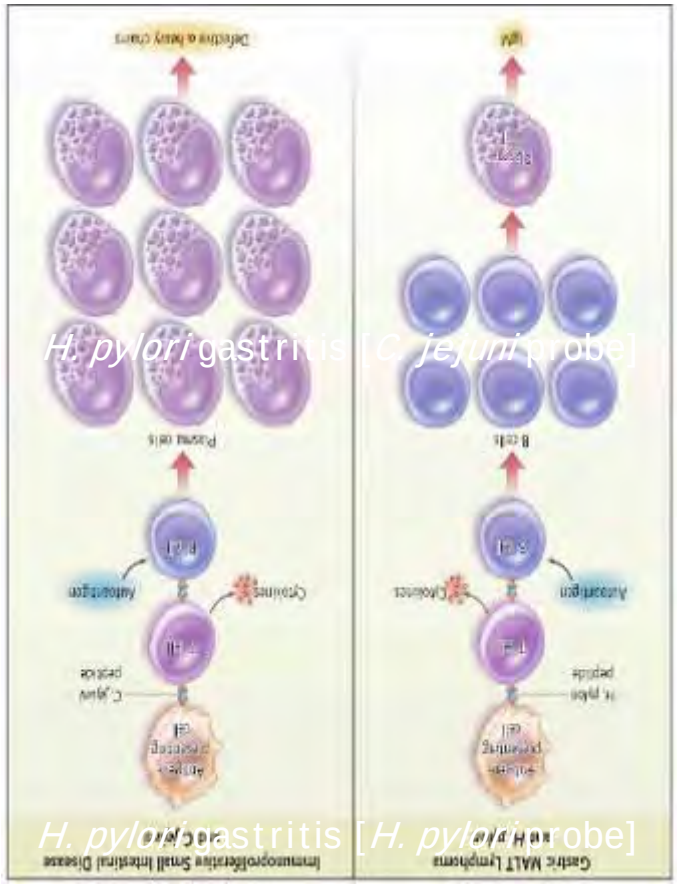
- Sensitivity



Microbiological investigations

In situ hybridization

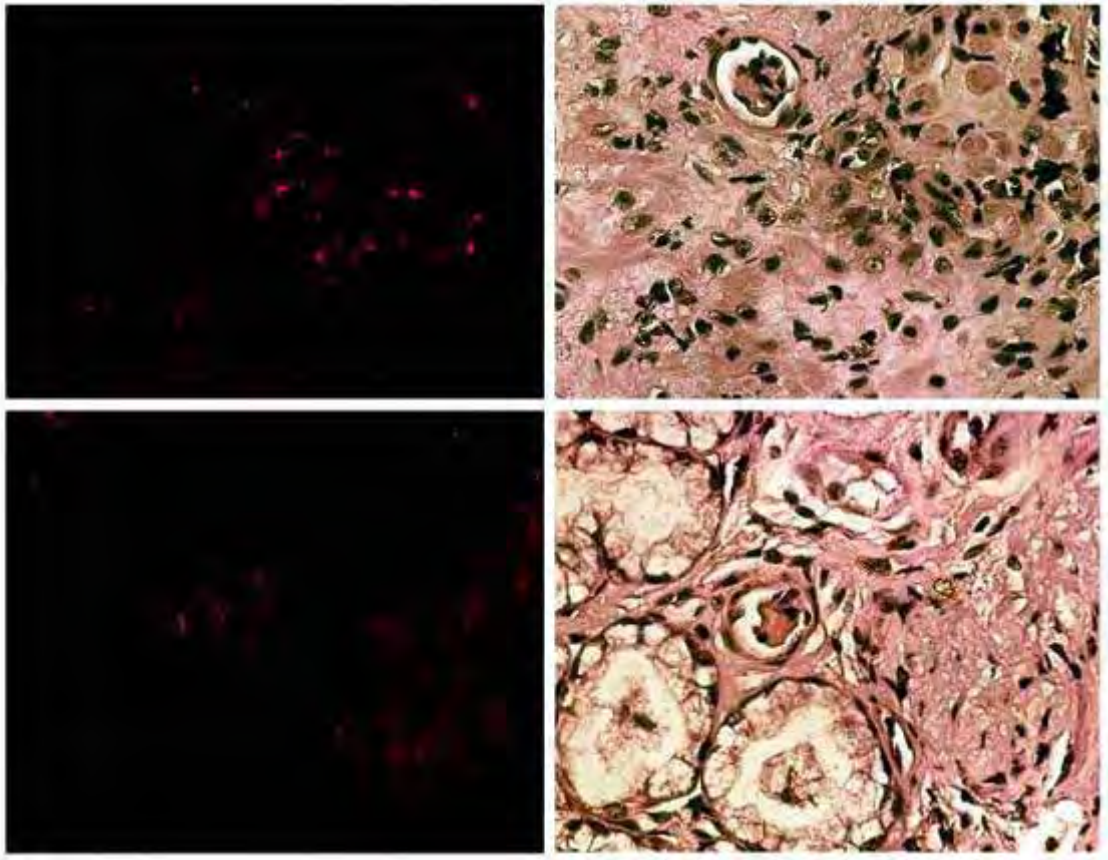
- Specificity



Microbiological investigations

In situ hybridization

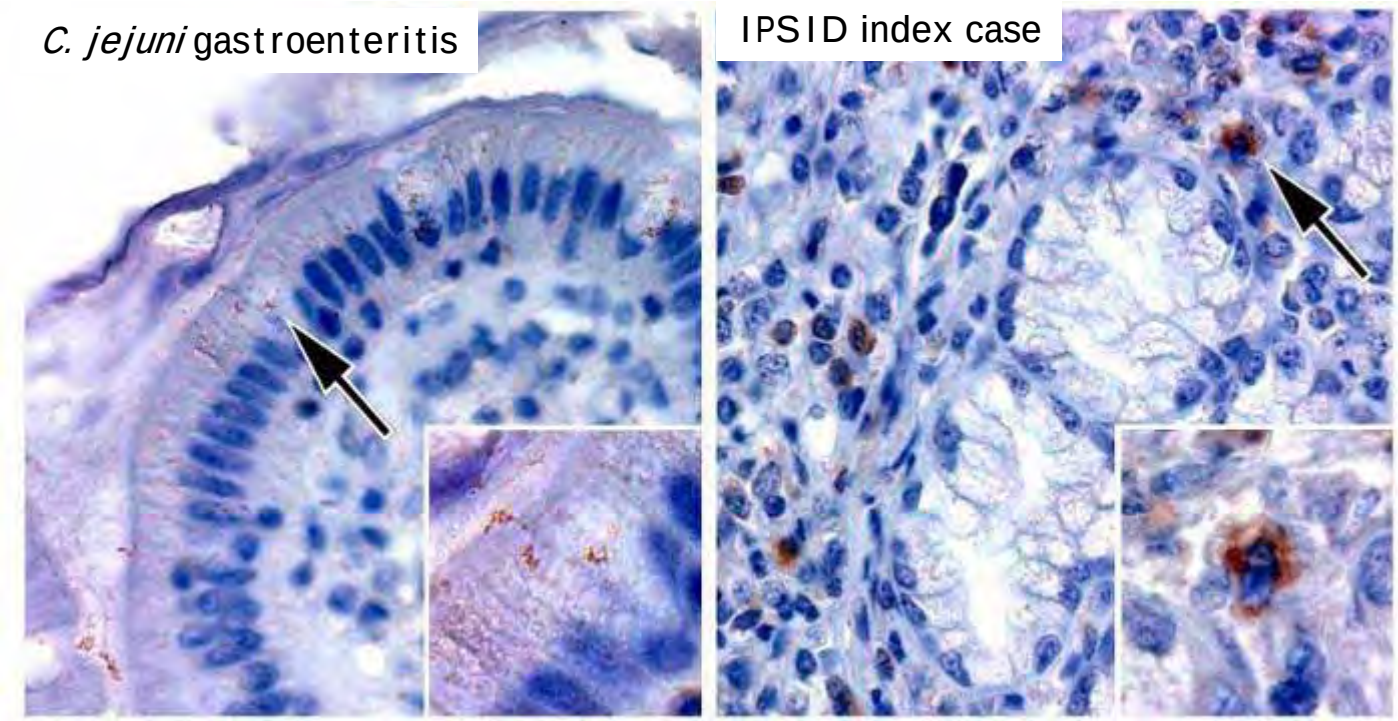
- Results



Microbiological investigations

Immunohistochemistry

- Results



Study of other cases of IPSID

Retrospective and monocentric study

- Material available from 6 cases of IPSID

- Archival paraffin-embedded jejunal biopsy specimens made at the time of the diagnosis (no prior treatment)

- Fixed in Bouin…

No amplifiable DNA (ß-actin control negative)

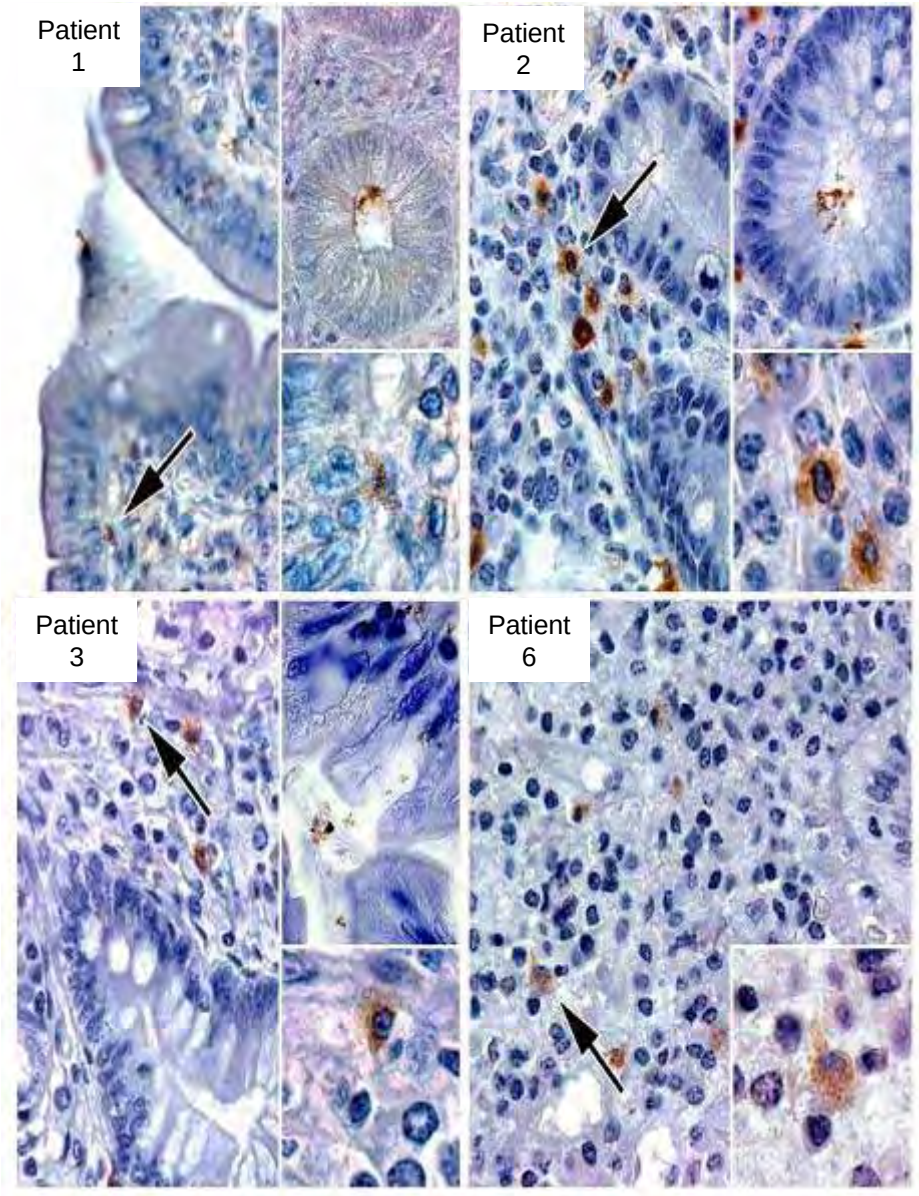
Study of other cases of IPSID

FISH and immunohistochemistry

Results of Fluorescence in Situ Hybridization and Immunohistochemical Assays of Biopsy Specimens from the Index Patient, Six Other Patients with Immunoproliferative Small Intestinal Disease, and Controls.*

Group and Diagnosis	FISH Results			Immunohistochemical Results	
	Bacterial 16S rDNA Probe	*Campylobacter jejuni* Probe	*Helicobacter pylori* Probe	*C. jejuni* and *H. pylori* Antibody	*H. pylori* Antibody
Controls					
C. jejuni enteritis	+	+	–	+	–
H. pylori gastritis	+	–	+	+	+
Normal duodenum from 10 patients	ND	–	–	–	–
Patients†					
Index patient, IPSID stage A	+	+	–	+	–
Patient 1, IPSID stage A	+	+	–	+	–
Patient 2, IPSID stage A	+	+	–	+	–
Patient 3, IPSID stage B	+	+	–	+	–
Patient 4, IPSID stage A	+	–	–	–	–
Patient 5, IPSID stage A	+	–	–	–	–
Patient 6, IPSID stage B	–	–	–	+	–

Study of other cases of IPSID



Implicate *C. jejuni* in 5 out of 7 cases of IPSID studied

From association to causality …?

Implicate *C. jejuni* in 5 out of 7 cases of IPSID studied

Fulfillment of Koch's Postulate

1. Is *C. jejuni* detectable in the infected host in the early stages of the disease ?
2. Is it possible to cultivate *C. jejuni* from the diseased tissue ?
3. Can *C. jejuni* trigger the disease in an animal model ?
4. If so, can *C. jejuni* be isolated from the diseased animal ?

In favor of a role for *C. jejuni* in IPSID development

❶ Epidemiological data

- Epidemiological similarities between the population chronically exposed to *C. jejuni* and the population in which IPSID occurs

- RR of *C. jejuni* infection in Morocco = 20 x Finland
(Infection, 1984)

- Chronic, relapsing and asymptomatic *C. jejuni* fecal carriage in children in developing countries in which IPSID is prevalent

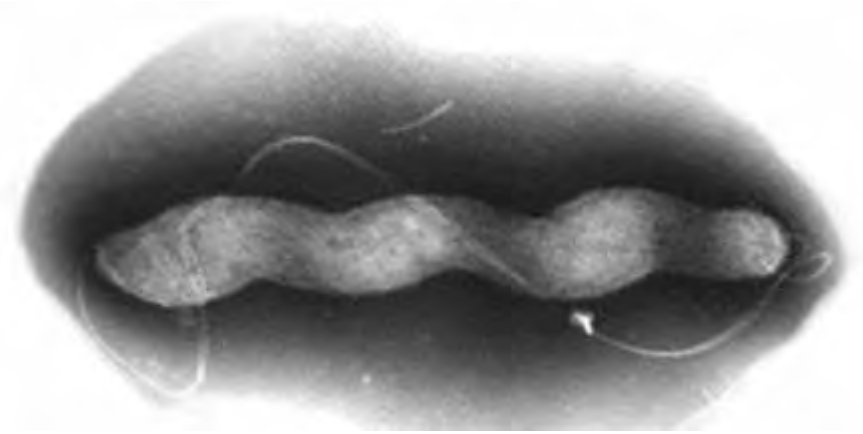
In favor of a role for *C. jejuni* in IPSID development

❷ Microbiological data

- *C. jejuni* diarrhea in the hours following chemotherapy for IPSID (Indian J. Gastroenterol. 1992)

- *C. jejuni* was discovered after IPSID was identified

- *C. jejuni* has to be cultured in a microaerophilic environment



In favor of a role for *C. jejuni* in IPSID development

❸ Therapeutic data

Antibiotics known to be active in IPSID are also active against *C. jejuni*

- Ampicillin
- Metronidazole
- Tetracycline
- Macrolides

In favor of a role for *C. jejuni* in IPSID development

❹ Similarities between gastric MALT lymphoma and IPSID as well as between *H. pylori* and *C. jejuni*

Phenotypic similarities	Gastric MALT lymphoma / IPSID
Distinct locations	Stomach / Small intestine
Phylogenetic relatedness	*H. pylori* / *C. jejuni*
Distinct niches	Stomach / Small intestine

Favors a causal role for *C. jejuni* in IPSID similar to that played by *H. pylori* in gastric MALT lymphoma

In favor of a role for *C. jejuni* in IPSID development

❺ Immunological data

Autoimmunity = frequently associated with MALT lymphomas

Hashimoto's thyroiditis	Thyroid MALT lymphoma
Sjögren's syndrome	Salivary gland MALT lymphoma
Anti-Lewis^b auto-antibodies	Gastric MALT lymphoma

C. jejuni = associated with autoimmune manifestations

Guillain-Barré's syndrome
Fiessinger-Leroy-Reiter syndrome

Lack of evidence for implicating *H. pylori*

Two cases of IPSID associated with *H. pylori* infection

(Lancet 1997, J. Clin. Gastro. 1998)

Significant or fortuitous association ?

No microbiological investigation except for *H. pylori*

Treatment used potentially active against *C. jejuni*

H. pylori association not confirmed in an Iranian study

(Arch. Iran. Med. 1999)

No significant association IPSID / *H. pylori* infection

H. pylori in 29 % of IPSID and 79 % non-ulcerous dyspepsia

H. pylori not found in our study

16S and specific PCRs

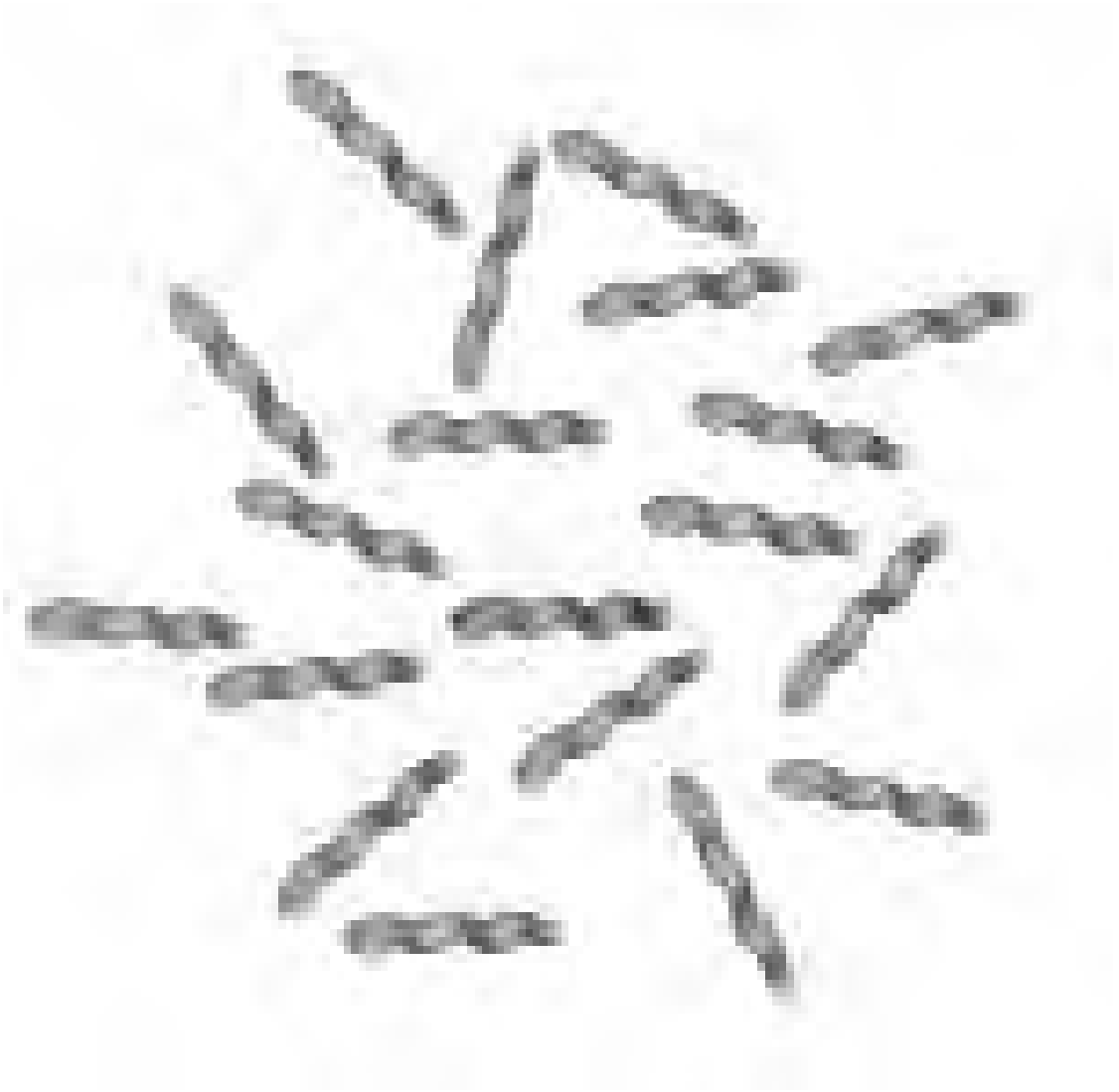
In situ hybridization

Immunohistochemistry

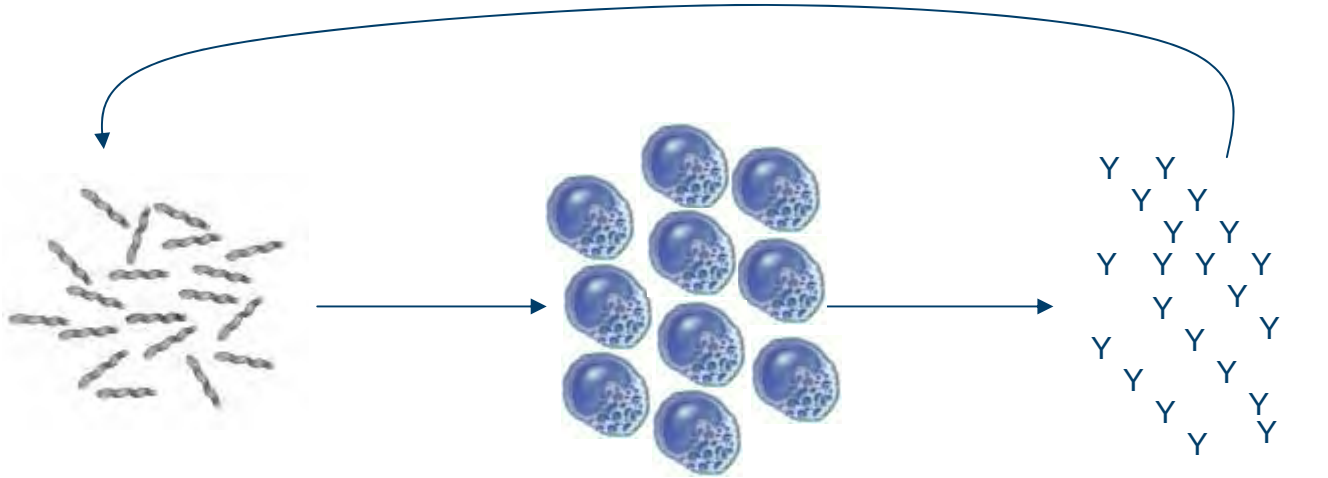
Pathophysiological model

≠ Direct lymphomagenesis
HTLV1
EBV, HHV-8

Antigen-driven lymphoproliferation



Pathophysiological model

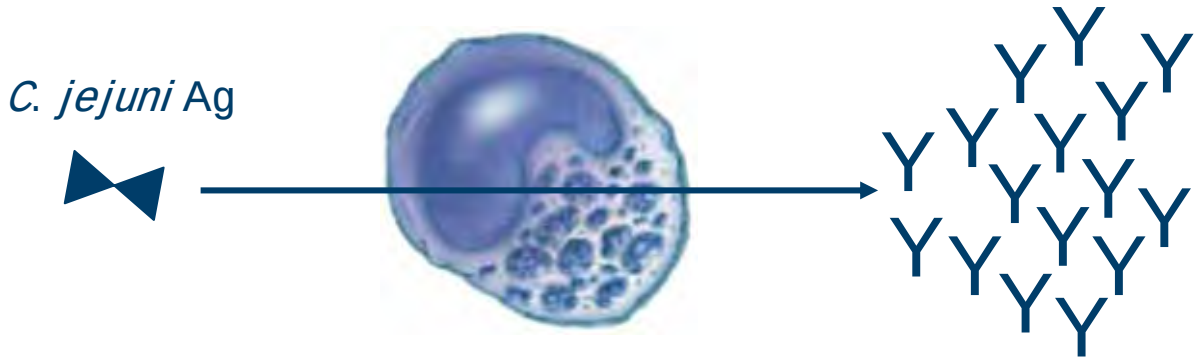


Chronic *C. jejuni* infection

Polyclonal lymphoplasmacyte proliferation

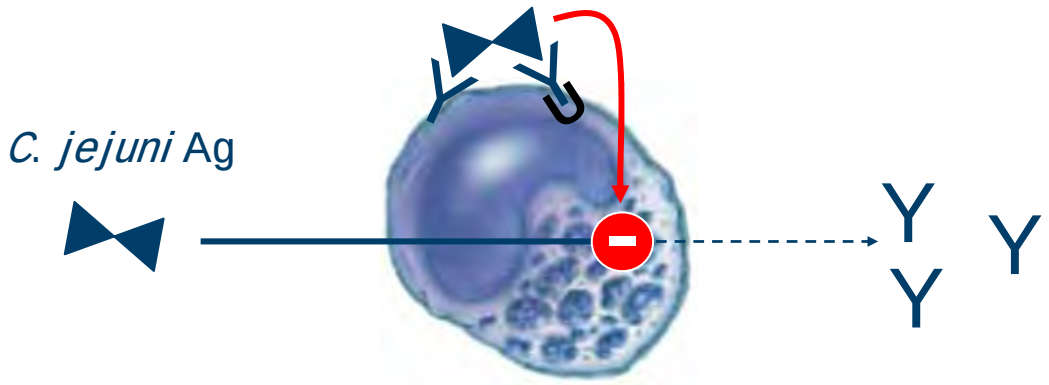
Polyclonal synthesis of IgAs

Pathophysiological model



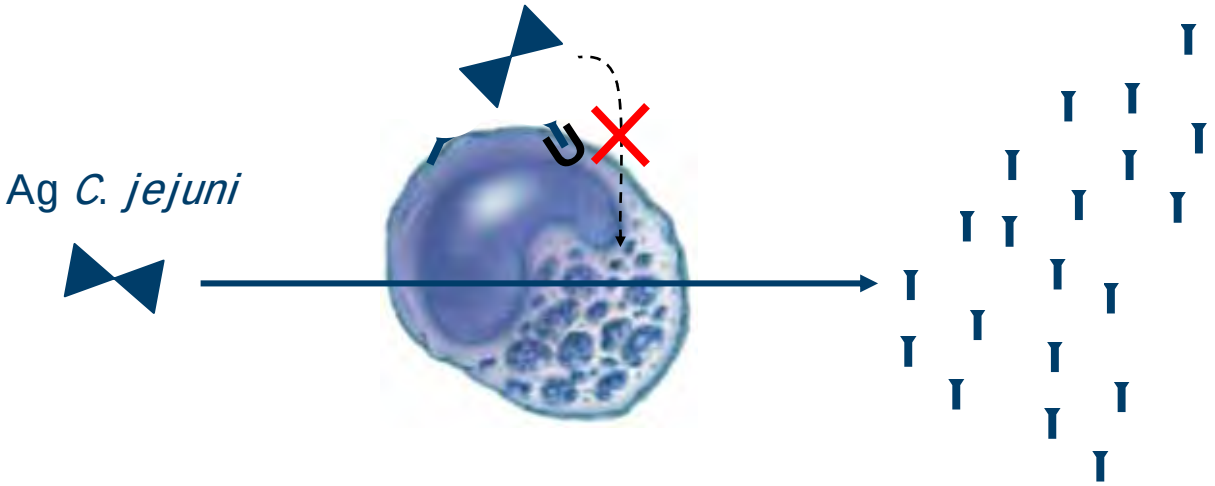
Chronic stimulation of mucosal immunity

Pathophysiological model



"Physiological" negative regulation of the IgA response
by antigen crosslinking of the surface Ig
and the Fcα receptor

Pathophysiological model



Absence of negative regulation in clones synthesizing a truncated IgA

Expansion of a clone responding to the mitogenic activity of the antigen but insensitive to the negative regulation

Pathophysiological model

Initial efficacy of the antimicrobial treatment eradicating the antigenic source

Expansion of a clone responding to the mitogenic activity of the antigen but insensitive to the negative regulation

Conclusions and perspectives

Prospective study

- “Quantify” IPSID association with *C. jejuni*
  - 16S and specific PCRs
  - *In situ* hybridization
  - In collaboration with Institut Pasteur International Network

- Optimal sample quality (Frozen biopsy samples)
- Culture under microaerophilic conditions
- Freezing of IPSID cells for further studies

Conclusions and perspectives

Pathophysiological studies

- Lymphoplasmacyte proliferation triggered by *C. jejuni* Ag ?
- T-dependent or T-independent response ?
- Selective advantage of the clone secreting the truncated IgA ?
  and/or
- Specific role of *C. jejuni* products ? (CdtB toxin induces DNA damages)

Application to other types of lymphomas

- *B. burgdorferi*-associated cutaneous MALT lymphoma
- *C. psittaci*-associated ocular adnexal MALT lymphoma
- ...

- HCV-associated splenic lymphoma with villous lymphocyte
- ...

Acknowledgements

René-Descartes Paris-5 University Medical School

Claire Poyart and Eric Abachin	Molecular biology
Philippe Pochart	*In situ* hybridization
Antoine Martin, Anne Lavergne	Histopathology
Loïc Guillevin	Cochin-Port Royal
Felipe Suarez, Olivier Lortholary	Necker-Enfants malades

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