Intestinal inflammation causes systemic genotoxicity

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Intestinal inflammation

- Inflammatory bowel diseases (Crohn’s disease, ulcerative colitis) and celiac disease

Chronic relapsing diseases involving many genetic and environmental factors resulting in increased risk for colorectal and small intestinal cancers among various extraintestinal manifestations

- DNA damage is beginning to be recognized as a feature of chronic inflammation
  - IBDs, rheumatoid arthritis, type 1 and 2 diabetes, chronic respiratory diseases, liver hepatitis/cirrhosis, dermatitis, and in cancer
Severity of symptoms

Two Atm-/- mice died due to treatment (one after cycle 2, one end of cycle 3)
Assays of genotoxicity

• Alkaline comet assay
  – Quantifies single and double strand breaks via the ‘Tail Moment’
  – Additional hOgg1 enzyme incubation will also detect oxidative base damage

• γH2AX immunostaining
  – Identifies DNA double strand breaks—Histone 2AX becomes phosphorylated in response to double strand breaks by ATM or ATM-like kinases

• In vivo micronucleus assay
  – Cytogenetic test to detect damage to chromosomes or mitotic apparatus of mammalian cells
Intestinal mucosal inflammation leads to genotoxicity to peripheral leukocytes.

Mean Olive Tail Moment

% Positive cells

MN-NCE/1000 NCEs
Genotoxicity in genetic models of spontaneous immune colitis

A

\[ \text{Gai}^{2/-} \quad \text{Gai}^{2/+} \quad \text{IL-10}^{-/-} \quad \text{IL-10}^{+/+} \]

\[ \times 100 \quad \times 200 \]

B

\[ \text{Mean Olive Tail Moment} \]

C

\[ \% \text{Positive Cells} \]

D

\[ \text{MNCE/1000 NCEs} \]
Intestinal inflammations causes systemic genotoxicity

Publication in Cancer Research. 2009 Jun 1;69(11):4827-34
Displayed on the cover of that issue

Press release by the Jonsson Comprehensive Cancer Center


Hundreds of webpages on the internet entitled like:
First ever link between intestinal inflammation and systemic genotoxicity
ATAXIA TELANGIECTASIA (AT)

Clinical manifestation:
- Autosomal recessive disease (1 in 40,000-100,000 people affected)
- Early-onset progressive cerebellar ataxia
- High incidence of tumors (1000 fold increase)
- Growth retardation
- Immunodeficiency

Biological markers:
- Chromosomal instability
- Hypersensitivity to radiation
- Imbalance in antioxidant levels and antioxidative enzymes
ATM$^{-/-}$ show elevated sensitivity to DSS
8-oxoguanine in distal colon of DSS-treated ATM−/− mice
Cytokine panel in peripheral blood

A: TNF-α/TBP
B: MCP-1/TBP
C: IFN-γ/TBP
D: IL-12b/TBP
E: TGF-β/TBP
F: IL-6/TBP
G: IL-10/TBP
H: IL-4/TBP

WT, ATM

Before cycle 1
After cycle 1
Before cycle 2
After cycle 2
Before cycle 3
After cycle 3
2 wks after cycle 3 cycle 3
4 wks after cycle 3 cycle 3

** *
Surface activation markers in peripheral blood

### Panel A

**Percent CD69+ T cells**

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<th>WT</th>
<th>ATM&lt;sup&gt;−/−&lt;/sup&gt;</th>
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### Panel B

**Percent CD44+ T cells**

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Conclusions

• Damage is mediated by inflammation derived RONS including nitrotyrosine and 8-oxoguanine

• Observed genotoxicity is proportional to clinical symptoms (treatment with DSS or age)

• Systemic distribution of cytokines

• Atm plays a critical role in maintaining genetic stability during intestinal inflammation
*Atm* deficient mice exhibit increased sensitivity to DSS-induced colitis characterized by elevated DNA damage and persistent immune activation.

- The lack of ATM causes further DNA damage and genetic instability (induced by byproducts of inflammation), along with a more potent immune response, possibly due to other pathways alerting and further activating the immune response.

- ATM therefore can be inferred to play a role in immunoregulation and maintenance of genetic stability during inflammation.
Which cell types are sensitive to DNA damage?
Sensitivity of various organs to genotoxicity

A

![Graph A](image)

B

![Graph B](image)
Genotoxicity to hepatocytes in mice with intestinal inflammation

A

B

C

D

Mean olive tail moment

Before treatment Water treated DSS treated

WT  Gαi2−/−

Mean olive tail moment

Before treatment Water treated DSS treated

WT  Gαi2−/−

Percent positive cells

Before treatment Water treated DSS treated

WT  Gαi2−/−

Mean olive tail moment

WT  Gαi2−/−

Percent positive cells

Before treatment Water treated DSS treated

WT  Gαi2−/−
Brain does not manifest DNA damage due to intestinal inflammation

A

Gai2\(^{+/−}\)

Gai2\(^{−/−}\)

x10

B

x63

C

Percent positive cells

Gai2\(^{+/−}\)  Gai2\(^{−/−}\)
Conclusions

- Intestinal inflammation causes both local and systemic genotoxicity to multiple cell types, which demonstrate different sensitivities and damage load.
- Decreased expression of ATM is not responsible for DNA damage as has been shown to be the cause in RA (not shown).
- Damage to lymphoid organs associate with severity of inflammation.

Inflammation associated systemic genotoxicity may explain higher occurrences of extraintestinal manifestations due to a systemic chronic inflammatory response.
Exploring mechanisms of inflammation-associated genotoxicity

Hypotheses:

Induction of DNA damage may require:

1) Presence of the cells at the site of inflammation, which then circulate systemically (does not explain damage to hepatocytes)

2) Presence of multiple cell types (classically activated monocytes/macrophages) which have the capability of releasing RONS during oxidative burst and damaging neighboring cells/themselves

3) TNF receptor-mediated signaling and its downstream mediators directly cause damage within the cell
Why TNF-α?

• TNF-α is highly upregulated in patients with IBDs (measured in mucosa, serum, stool) and implicated in pathogenesis
  – Chronic liver hepatitis
  – Metabolic diseases
  – Rheumatoid arthritis and other autoimmune disorders
• Systemic and persistent presence may explain systemic genotoxicity
TNF-α is sufficient to induce genotoxicity *in vivo* in wildtype mice.
Profile of genotoxicity is similar to IBD models
TNF-α causes DNA damage in primary splenic T-cells

γH2AX foci formation in non-apoptotic cells

Apoptotic cells (PI+, TO+)

The graphs show the percentage of positive cells and dead cells over time for different concentrations of TNF-α.

- **Saline**
- **1 ng/mL TNFα**
- **10 ng/mL TNFα**
- **100 ng/mL TNFα**
Co-administration of TNF-α and IL-1β results in sustained DNA damage *in vivo* and *in vitro*.

**in vivo**
- Percent Positive Cells
- Saline
- IL-1β
- IL-1β + TNF-α

**in vitro**
- % positive cells
- Saline
- 10ng/mL IL-1β
- 10ng/mL IL-1β + 10ng/mL TNF-α

Cell viability
TNFR1\(^{-/-}\)/TNFR2\(^{-/-}\) mice are less sensitive to DSS-induced colitis
Genotoxicity in TNFR1−/−/TNFR2−/− mice after injection with TNF-α
Genotoxicity in peripheral organs of TNFR1⁻/⁻/TNFR2⁻/⁻ mice 1hr after TNF-α injection
TNF-α mediated signaling via the TNFR involves activation of proinflammatory cell survival/proapoptotic genes
Effect of NF-κB inhibitors on TNFα-induced genotoxicity

![Bar chart showing the effect of NF-κB inhibitors on TNFα-induced genotoxicity. The x-axis represents different treatments: Saline+Saline, Bortezomib+Saline, Emetine+Saline, Chromomycin A3+Saline, Saline+TNFα, Bortezomib+TNFα, Emetine+TNFα, Chromomycin A3+TNFα. The y-axis represents the percent positive cells. The chart indicates a significant increase in genotoxicity after injection with TNFα compared to saline treatments.](Image)
Decreased ATM expression is not responsible for increased genotoxicity in genetic models.
Conclusions

• TNF-α is sufficient to cause genotoxicity in wildtype mice, which is reduced when administered NF-κB inhibitors
• TNF-α + IL-1β induces prolonged genotoxicity without successful repair even after 24 hrs

• TNF-α administration to splenic T-cells is sufficient to cause DNA damage in the absence of other cell types
• DNA damage and cytotoxicity are both increased when T-cells are co-incubated with CD11b+ monocytes

• TNFR mediated signaling is heavily involved in DNA damage resulting from DSS-induced colitis
• TNF-a may increase intracellular ROS and cause stronger TNFR mediated signaling—DNA damage may be a side effect of this process
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