Poster No.55

New therapeutic option in autoimmune diseases to prevent the release of soluble CD95L

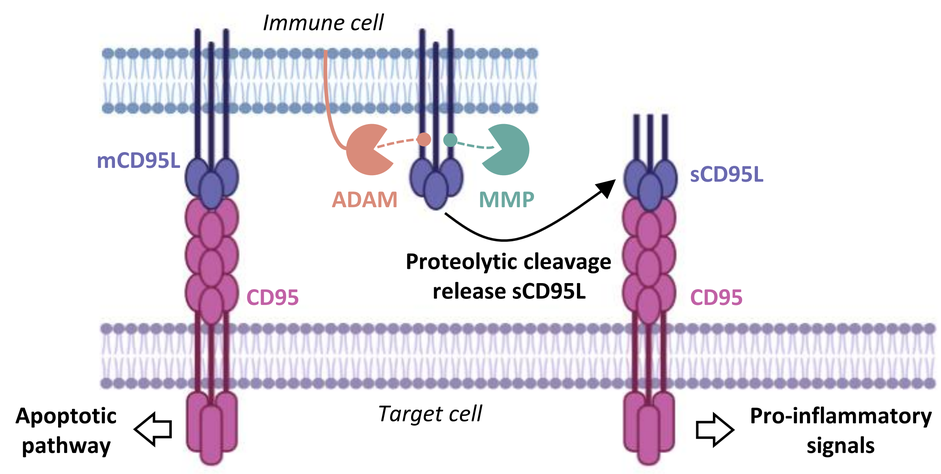
LAUNAY Marie[1], HUOT-MARCHAND Sarah[2], KOTAICH Farah[3], BLANCO Patrick[2], LEGEMBRE Patrick[3], DEVEL Laurent[1]

[1]**Université Paris-Saclay**, *CEA, INRAE, Médicaments et Technologies pour la Santé (MTS), SIMoS, France.* [2]**ImmunoConcept**, *CNRS UMR 5164, Université de Bordeaux, France.* [3]**UMR CNRS 7276,** INSERM U1262, CRIBL, *Université Limoges, France*

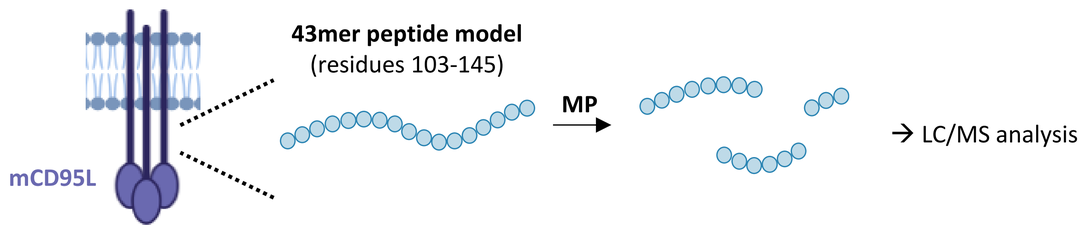
https://doi.org/10.17952/38EPS.2026.P055

1 Pathological context

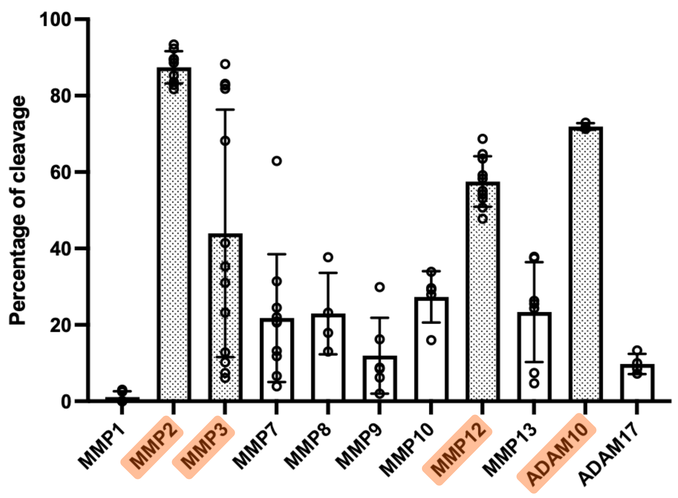
Autoimmune diseases affect approximately 10% of the population, with elevated levels of soluble CD95L (sCD95L) notably observed in systemic lupus erythematosus (SLE). While membrane-bound CD95L (mCD95L) induces apoptosis and contributes to immune regulation, its proteolytic cleavage by metalloproteases (MPs) generates sCD95L, shifting signaling toward a pro-inflammatory response. The mechanisms and precise cleavage sites involved in MP-mediated CD95L processing remain unclear. To investigate this process, we designed a peptide model corresponding to the CD95L cleavage region. This study aims to identify the cleavage sites and characterize the role of MPs in CD95L processing. In addition, we evaluate an antibody-based strategy to prevent CD95L cleavage, in order to preserve its apoptotic function and limit inflammatory signaling.



2 MPs identification & cleavage sites



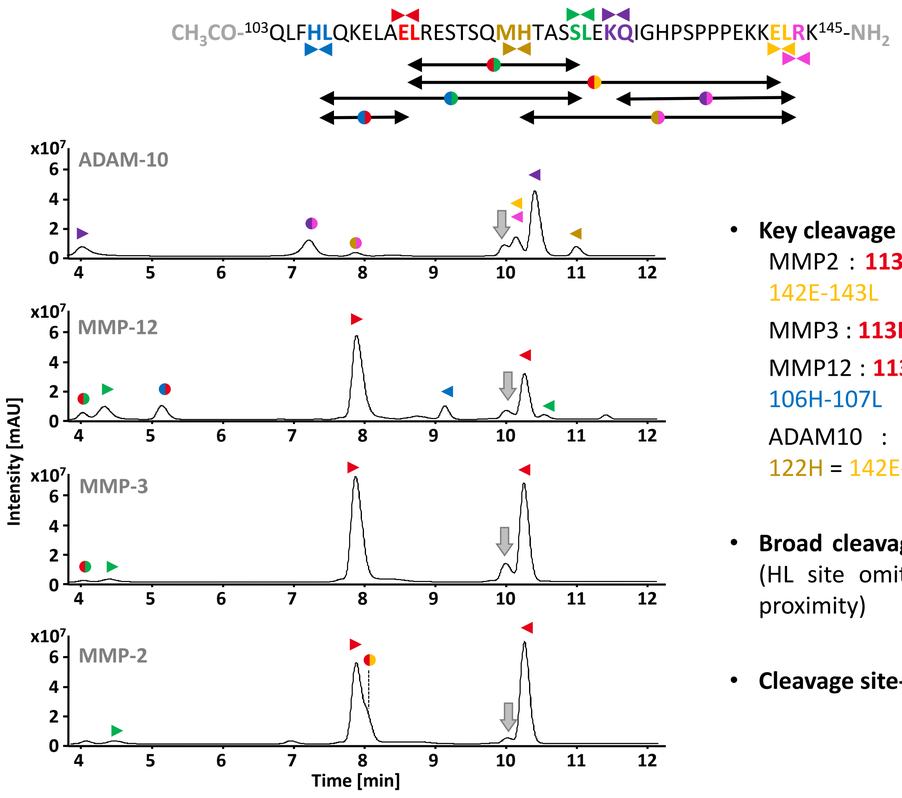
- **Most active MPs in peptide processing**



Metalloprotease families involved:

- **Matrix Metalloproteases (MMPs):** conserved cleavage sites within the family (**113E–114L** & **126S–127L**).
- **A Disintegrin And Metalloproteases (ADAM):** preferential cleavage at a distinct **129K–130Q** site.

- **Cleavage site identification**

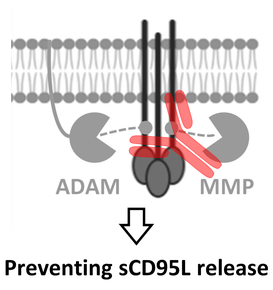


- **Key cleavage position :**
  MMP2 : **113E–114L** >> **126S–127L** > 142E-143L
  MMP3 : **113E–114L** >> **126S–127L**
  MMP12 : **113E–114L** >> **126S–127L** = 106H-107L
  ADAM10 : **129K–130Q** >> 121M-122H = 142E-143L = 143L-144R

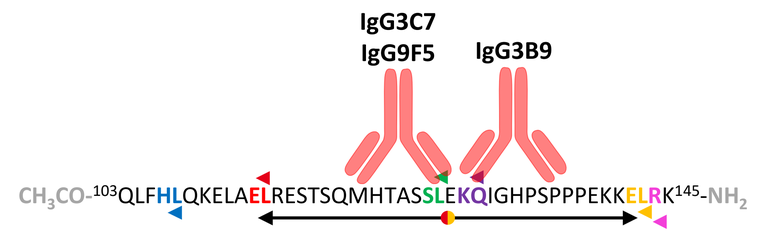
- **Broad cleavage diversity for MMP12** (HL site omitted due to membrane proximity)

- **Cleavage site-rich region**

3 New strategy to block sCD95L release

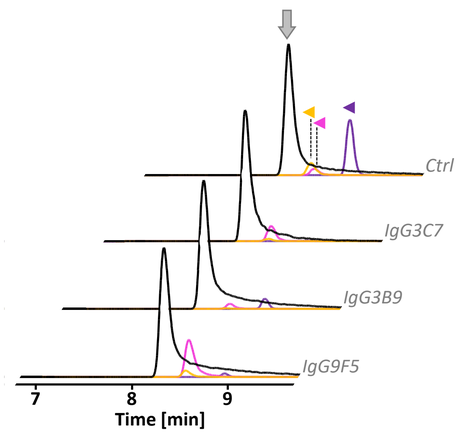


→ **Screening of three antibodies (IgG)**



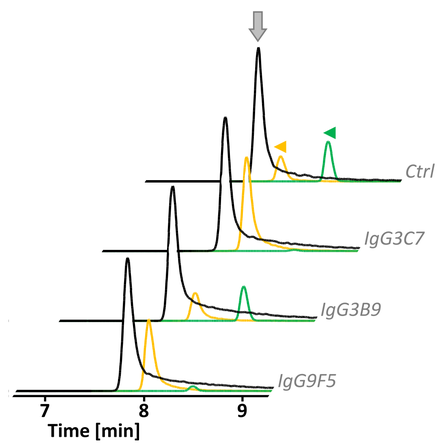
For MMPs, cleavage at 113E–114L masked protection at the 126S–127L epitope. The 113E–114L → GG mutant was used to assess protection at this site

- **Against rhADAM-10 processing (n=3)**



✓ **IgG3C7** and **IgG9F5** provide stronger protection against cleavage at **129K–130Q** site

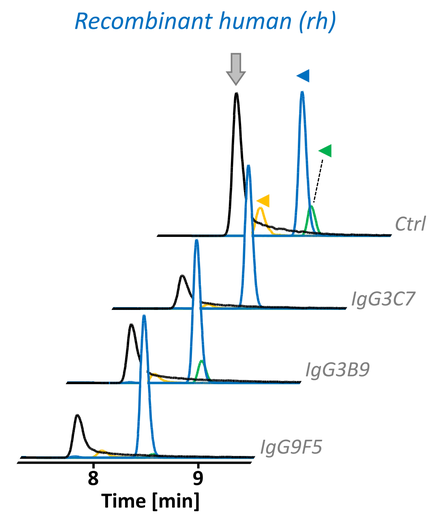
- **Against rhMM-2 processing (n=3)**



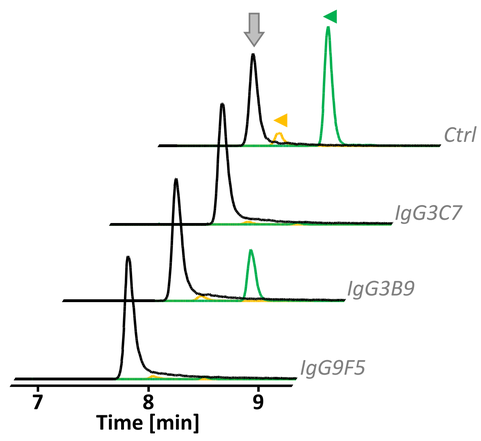
✓ **IgG3C7** provides stronger protection of the **126S–127L** site.

- **Against MMP-12 processing (n=3)**

Recombinant human (rh)

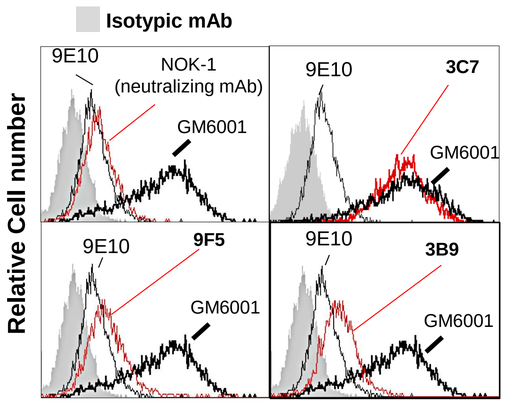


Recombinant mouse (rm)



✓ **Differential processing** between rhMMP-12 and rmMMP-12, consistent with **low homology (61%)**
✓ **IgG3C7** provides stronger protection of the **126S–127L** site.

- **Evaluation on cell**



- Assessment mCD95L expression levels in cells (increasing towards the right)
- GM6001, a broad-spectrum metalloprotease inhibitor, blocks mCD95L cleavage
- IgG3B9 and IgG9F5 show low mCD95L expression compared to GM6001
- **IgG3C7**: increased mCD95L levels, comparable to GM6001

4 Future perspectives

✓ **IgG3C7 :**
- Emerged as the **best candidate for preventing cleavage** within the cleavage site-rich region
- Has shown a **protective effect in cell-based assays**
- Currently **being tested in murine models** of autoimmune disease to prevent mCD95L cleavage

→ **Evaluate protection against rmADAM-10 (96% homology with rhADAM-10)**

→ **Identifying MPs in murine models of autoimmune disease:**
- Use a broad-spectrum substrate to identify metalloproteolytic activity
- ELISA assays to **quantify** MMP2, MMP3, MMP12, and ADAM10 **levels across** different disease stages
- **Activity-based probes** used to capture and identify **active metalloproteases** at different pathological stages.

Collaborations

SIMoS · MTS Médicaments et Technologies pour la Santé · anr agence nationale de la recherche ANR-24-CE18-2934 · ImmunoConcEpT · cnrs

[1]Legembre P, Blanco P, Jean M. WO2023161412. 31 août 2023.
[2]Devel L et al. Role of metalloproteases in the CD95 signaling pathways. Frontiers in Immunology. 05 dec 2022;13:1074099.