Comments and Suggestions for Authors

Reviewer 2

The manuscript by Saulle et al. describes the roles played by endoplasmic reticulum aminopeptidase 1 and 2 (ERAP1 and 2) in the anti-pathogen immune response, specifically in the cases of infection by CMV, influenza virus, HPV and HIV. There have been few reviews on ERAPs recently but none that covered this topic as well and as specifically as this one. The review is well-organized and studies are cleverly discussed. The analysis of the litterature is thorough and provides a panoramic landscape of the field.

Here are some minor considerations:

1. Too many references are reviews instead of original findings. This should be addressed. References format is not uniform and dates are not in English.

We apologize for the mistake. The bibliography has been properly reviewed

2. One format of citing X. XXXX et al., should be chosen and standardised throughout the text.

Changes were made accordingly.

3. Table 1 could be 'prettier' if formatted differently

Here is the new version of the table

(A)

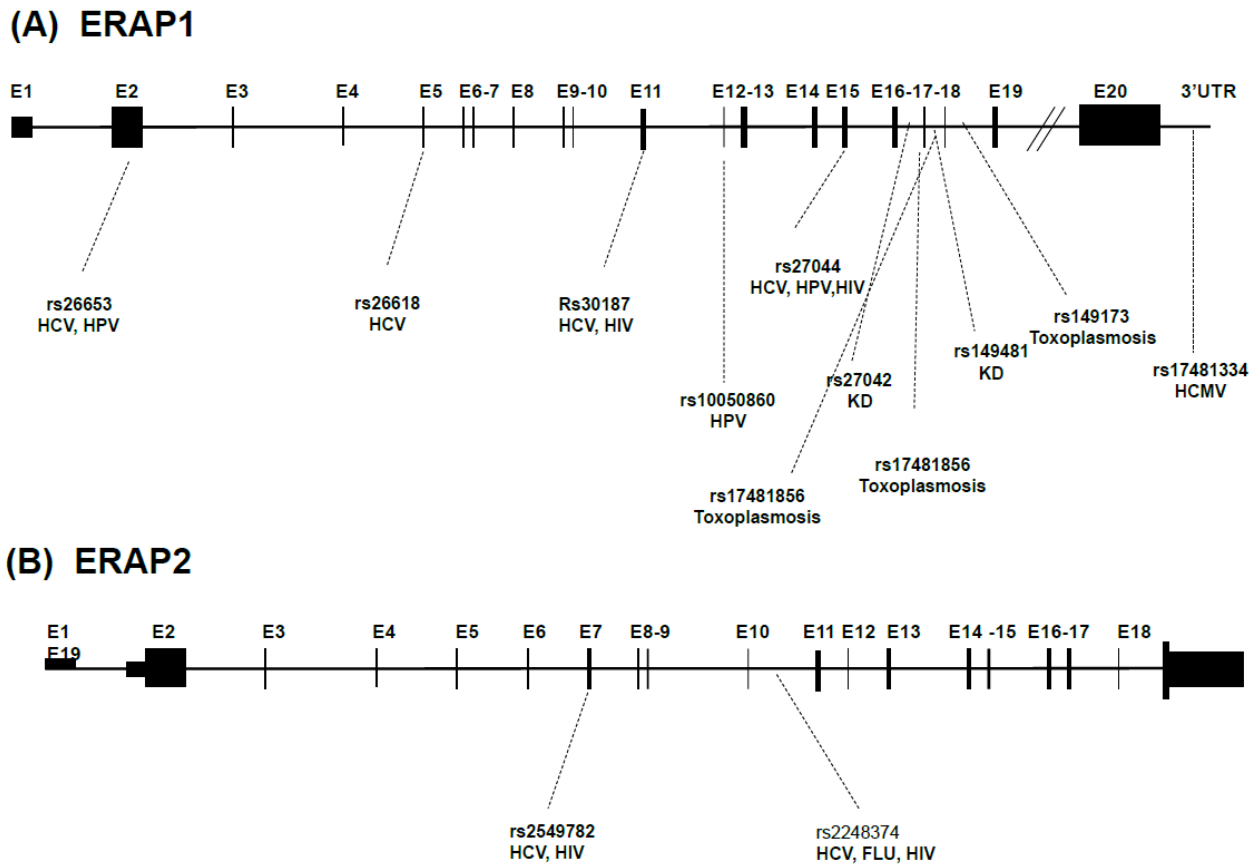
4. ERAP1 SNPs	Region	Variation	Infectious disease(s)
rs30187	Exon 11	K528R	HCV, HIV
rs27044	Exon 15	Q730E	HCV, HIV, HPV
rs10050860	Exon 12	D575N	HPV
rs26618	Exon 5	M276I	HCV
rs26653	Exon 2	P127R	HCV, HPV
rs17481856	Exon 17	L848L	Toxoplasmosis
rs17481334	3’ UTR	None	HCMV
rs149481	Intron 17	None	KD
rs27042	Intron 16	None	KD
rs149173	Intron 18	None	Toxoplasmosis

(B)

ERAP2 SNPs	Region	Variation	Infectious disease(s)
rs2549782	Exon 7	K392N	HCV, HIV
rs2248374	Intron 10		HCV, Flu, HIV

5. Figure 1 is low resolution and should be referred to earlier in the text. A subfigure containing the SNPs described in the text that are not related to infectious diseases could be added. A 3D structure image or a simple representation of the proteins could help with the visualisation of the reported mechanisms.

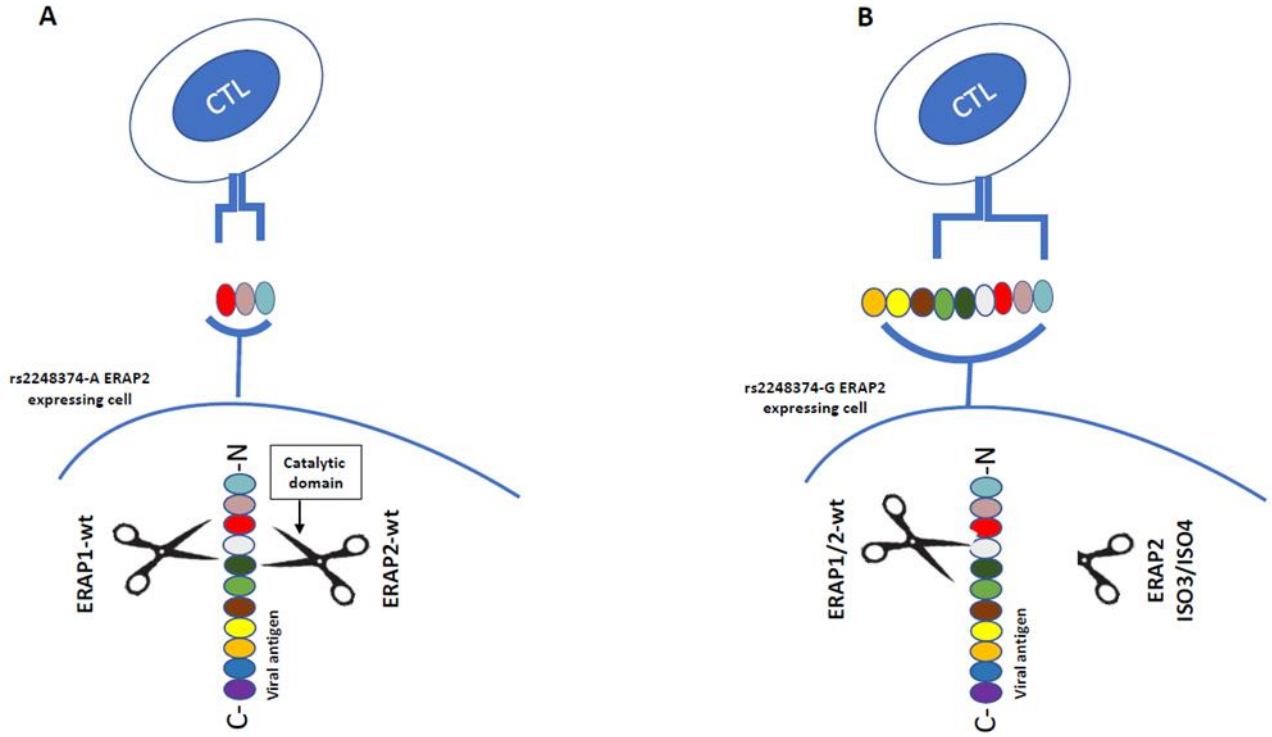
According to the reviewer suggestion Figure 1 has been referred earlier in the text (Line 105). And its resolution has been improved.



As for the subfigure containing the SNPs described in the text that are not related to infectious diseases, we believe that it would be better to focus the reader attention exclusively on those variants which have been associated to infectious diseases as this is the focus of our review.

Concerning the 3D structure of the protein we suggest a link within the text to view the deposited structure of ERAP1: "A 3D structure of ERAP1 is deposited at the following link: http://www.rcsb.org/structure/2YD0 deposited in PDB database by : Vollmar, M., Kochan, G., Krojer, T., Ugochukwu, E., Muniz, J.R.C., Raynor, J., Chaikuad, A., Allerston, C., von Delft, F., Bountra, C., Arrowsmith, C.H., Weigelt, J., Edwards, A., Knapp, S."

6. Figure 2 could be improved. Are the peptides longer in case B when ISO3 and 4 are expressed? Is there still some catalytical activity? why is half of a scissor drawn?
   The figure has been changed according to the reviewer suggestions. Peptides should be longer because, in our hypothesis, ISO3 and ISO4 should prevent wt ERAP1 and 2 activation (dominant negative effect), thus avoiding the trimming of the antigen. Half of the scissor was draw to simulate the loss of the catalytic site in ISO3 and ISO4. To better represent such conditions, both blades have now been removed from the figure to represent ISO3 and ISO4.



7. The + in CD8+ T cells should be "superscripted".
   Changes were made accordingly

Here are some other comments:

8. Line 29: Other factors than virulence and host cell immune response may impact on the onset of infectious disease. The sentence 'The potential onset of infectious disease depends on the virulence of the microbe and the efficacy of the host immune response which envisages the coordinated action of all the elements responsible of defenses against pathogens, from physical barriers to multiple components of the innate and adaptive immune systems' could be modified not to exclude other possibilities.
   The observation is correct. The sentence has been changed by adding “among others” not to exclude other factors possibly involved: ” The potential onset of infectious disease depends on the virulence of the microbe and the efficacy of the host immune response, among others, which envisages the coordinated action of all the elements responsible of defenses against pathogens, from physical barriers to multiple components of the innate and adaptive immune systems.”

9. Line 42: 'above mentioned changes' refer to the abstract and could be repeated in the main text.

The sentence has been rephrased: “Based on these premises, this review aims to provide an overview on the role played by Endoplasmic reticulum aminopeptidase 1 (ERAP1) and 2 (ERAP2), which are primarily responsible for the generation of the antigenic repertoire presented by MHC I molecules, in the control of susceptibility as well as progression of different infectious diseases”

10. Line 46: add 'cellular' between 'human' and 'immune'
    Changes were made accordingly
11. Line 56: [In humans, ... aminopeptidases] should mention the original papers.
    Changes were made accordingly
12. Line 61: [The trimming ... presentation] add reference to original discovery.
    Changes were made accordingly
13. Reference 1.2.3.4..... are reviews
    Changes were made accordingly
14. Line 68. Phrasing is confusing in:[The crystal structure of ERAP1 shows a 4-domain protein whose catalytic site is located in domain II a cavity, covering areas of domains II and IV, is the substrate-binding site.]

The reviewer is right. Part of the sentence was missing therefore it was completely rephrased: “The crystal structure of ERAP1 shows a 4-domain protein whose catalytic site is located in domain II. Domain I caps off the active site and provides binding sites for the amino terminus of a substrate peptide; domain III acts like a hinge enabling conformational changes between domains II and IV; domain IV has a concave structure fronting the active site”

15. Line 90: Ambiguous phrasing: confirm that it may. [A more recent work confirmed that also ERAP2 may be secreted by human macrophages triggered by inflammatory stimuli]
    “May be” has been changed with “Is”

16. Line 138: Phrasing.
    Done

17. Line 150: could you be more specific? [Such observation suggests that, despite NMD, transcripts derived from both haplotypes could confer fitness advantages, possibly in diverse milieus]
    The sentence has been rephrased in this way: “The maintenance of both haplotypes with the same incidence suggests that, despite NMD, even transcript(s) derived from HapB could confer fitness advantages, possibly in particular milieus”

18. Reference 38 and 39 should cite original findings.
    Changes were made accordingly

19. Section 2.1 ERAPs and HCV. The existence of efficient anti-HCV drugs is worth mentioning.
    The reviewer is right. A brief sentence enlightening this crucial aspect has been added to the manuscript: “Fortunately, the availability of highly efficient direct-acting antivirals has revolutionized the treatment landscape with over 95% cure rates as accurately reviewed in (42).”

20. Line 245: The role of ERAP2 on ERAP1 function should be further (and earlier) discussed? [In addition, given the role of ERAP2 as a promoter of ERAP1 activity - through the formation of heterodimers with increased trimming efficacy - it has been observed that combination of rs26618-C/rs2248374-A on the two genes also provides a greater risk of susceptibility to HCV chronicity in Han Chinese population (46).]
    The observation is pertinent. Therefore, a further discussion on ERAP1 and 2 interaction has been added in the *Biological functions of ERAP1/2* paragraph: “Contrariwise, ERAP2 presents a marked

preference for the positively charged arginine and lysine residues situated at the N-terminal and shows a greater efficiency towards shorter peptides that ERAP1 processes poorly (4,11). Notably, in 2005 L. Saveanu et al., demonstrated that ERAP1 and ERAP2 can physically interact forming a heterodimer (7). Ten years later, using engineered ERAP1–ERAP2 complexes, I. Evnouchidou and colleagues verified that heterodimer formation changes enzymatic features and increases peptide-trimming efficiency, thus allowing the generation of a wider and more immunogenic antigenic repertoire (8). Indeed, the complementary activity of the two aminopeptidases leads to the generation of a variegated antigenic repertoire, which may be altered in individuals lacking either protein (12). Overall, it has been demonstrated that the structural changes made by ERAP1 and ERAP2 represent a key step in mature antigenic epitope generation (12)".

21. Line 254 missing space

The typo has been corrected

22. Line 264: the onset of the flu?

The sentence has been rephrased: "Influenza virus is of the etiological agent of influenza"

23. Line 270: The sentence [An effective vaccine is available...among the elderly.] may be misleading: even though vaccination is recommended (and should be) and is indeed beneficialm the sentence does not take into account that each year the flu vaccines are different, as is their efficacy.

    The sentence has been rephrased in this way: "Every year a new mostly effective vaccine is available and recommended, in order to prevent the disease in healthy adults and to reduce infection severity among the elderly"

24. Line 292: standardise FLU or flu.

Influenza virus has been identified by the acronym Flu all throughout the paper

25. Line 274: The sentence should refer to original findings.

Changes were made accordingly

26. Line 337-338 Which country of the [Institute of Medicine and the National Vaccine Advisory Board]

Washington, USA. This information has been specified in the text

27. Line 346-347 ref 60 and 61 are reviews.

Changes were made accordingly

28. Line 374: Can you define the nomenclature of AA and GG individuals.

In order to better define the nomenclature of AA and GG subjects the paragraph has been revised: ". Indeed, miR-UL112-5p directly targets the 3' UTR of ERAP1-A SNP variant, thus downregulating its mRNA expression and altering the presentation of many HCMV-derived antigenic peptides to CD8+ T cells (71). Notably, the authors identified the rs17481334-G SNP variant, naturally occurring in the ERAP1 3' UTR, which disrupts the consensus sequence for HCMV miR-UL112-5p and, in turn, prevents ERAP1 targeting and degradation".

29. Line 391: missing space

Done

30. Line 393: Its not [It's...]

Done

31. Line 405: APM and HLA abbreviations could have been defined ealier, line 52 or 240?

According to the reviewer suggestion the acronyms APM and HLA have been introduced earlier: lane 53 and lane 252, respectively.

32. Line 408: Define LMP

LMP stands for low molecular weight peptide as now it has been explained in the text (lane 409)

33. Line 448: [highly] could be removed

Changes were made accordingly

34. Line 492: [shered] is shared

    The typo has been corrected

35. Line 513: phrasing could be better.

The sentence has been rephrased: “The presence of the virus causes a severe immunodeficiency condition, characterized by CD4+ T cell loss responsible for the onset of infections and the development of malignant tumors that otherwise rarely infect human beings to cause illness (105)”

36. Line 519: latency can also be explained by other independant mechanisms, the sentence: [The proviruses may remain transcriptionally-inactive for a long time and this event explains the latency of HIV ininfected people.] should be less affirmative.

The sentence has been rephrased according to the reviewer observations: “The proviruses may remain transcriptionally-inactive for a long time and this event is partially responsible for the latency of HIV in infected people”

37. Line 530: are these findings consistent with the fact that HLA-B57+ patients often exhibit a strong and protective CTL response?

The observation is pertinent and reliable and was added to the manuscript

38. Line 551: Nevirapine

Changes were made accordingly

39. Line 570: rhERAP2 = rhesus?

    Rh stands for recombinant human as now specified in the manuscript

40. Line 609: underline.

The typo has been corrected