RESPONSES TO REVIEWER'S COMMENTS (00503062)

Q: I wonder if there are some studies on cell-mediated immunity, namely, T-cell responses to glycated proteins. It would be interesting if T-cell recognition (T-cell epitope) is changed after protein glycation.
A: While expressed inducibly on T-cells of healthy subjects upon TCR activation, the receptor for advanced glycation end products (RAGE) is constitutively synthesized in diabetics' T-cells, and a role for RAGE in the adaptive immunity has been proposed. In has been demonstrated that ovalbumin (OVA) modified with AGEs (pyrraline), but not native OVA, induces SR-A mediated uptake of the antigen by dendritic cells and enhances $CD4^+$ T-cell immunogenicity and potential antigenicity of OVA. This data together with relevant references (ref. Nos. 146-149] are now included on page 14, lines 5-11 of the revised manuscript. Thank you for this question contributing to the fullness of our review. To the best of our knowledge, to date no one has demonstrated glycation of TCR or other T-cell epitopes.

Q: Page 15, line 10. "Least but not last" reads "Last but not least".
A: "Least but not last" was changed to "Last but not least" (now on page 18, line 17).

Q: Page 18, line 9. "has and" reads "and has".
A: "has and" was replaced with "has an" but not with "and has", as suggested by the referee, because this is what we really mean (now on page 21, line 16).

Q: Page 19, line 3 from bottom. "This results" reads "These results".
A: We did not change "This results" to "These results". Instead, to avoid confusion we merged the two sentences by using "resulting" as a conjunction (now on page 23, line 5).

Q: Page 43, Figure 5 legend. Flows of protein "oxidation and nitration" free adducts are not shown in Figure 5 although they are described in Figure 5 legend. Reconsider description of the legend.
A: The redundant text "oxidation and nitration" is omitted from the Figure 5 legend (now on page 61).

Q: Page 43, Figure 7, 8 legends. Authors should describe briefly the experimental conditions such as sample numbers examined in the legends of Figures 7 and 8.
A: In fact, in the legend to Figure 7 of the original manuscript we provided data on the sample size on page 43, lines 11-13 as follows: "Three vials of each drug were analyzed by competitive ELISA in duplicates, and the pooled results were presented as means ± SD." The text is left unchanged in the revised manuscript (page 63, lines 4-6 from bottom). Regarding Figure 8, if we provide detailed information on the experimental conditions, too much text has to be added to this figure. Therefore, in the revised manuscript we preferred to refer to our previous work by inserting the sentence "For detailed information on the experimental conditions see ref. No. 211" (page 64, lines 1-2 from bottom).

Q: Check fonts for "AGEs-BSA" in y-axes of Figures 7 and 8. They are different.
A: Fonts for "AGEs-BSA" in the y-axes of Figures 7 and 8 have been equalized in the revised

manuscript (Figure 7, page 63).

Q: Figure 9. In the figure, "T cell naïve" should be "naïve T cell", and "T cell active" should be "activated T cell".
A: Changes have been introduced in Figure 9 of the revised manuscript according to the referee's remark (Figure 9, page 65).

RESPONSES TO REVIEWER'S COMMENTS (00502947)

Q: Is there any direct evidence that the Maillard reaction gives rise to immunogenicity?
A: Antibodies against AGEs have been detected in healthy subjects and under various diseases conditions as well, which points to the immunogenicity of the Maillard reaction. Another line of experiments shows that immunization of animals with AGEs-modified proteins, DNA and LDL elicits higher titer Abs formation as compared to immunization with non-glycated antigens. This information is now included in the revised manuscript on page 14, line 7 (from bottom) to page 15, line 1 (from top). In fact, in the original manuscript we discussed on the link between Maillard reaction and immunogenicity under the heading "ROLE OF THE IMMUNE SYSTEM IN THE ANTI-GLYCATION DEFENSE", where respective references (ref. Nos. 131-137) were provided. Assuming that the referee asks this question because we were not clear and convincing enough, we now cite additional articles (ref. Nos. 155-164 in the revised manuscript) evidencing the immunogenicity of the Maillard reaction.

Q: Has anyone try to inhibit AGE formation and prevent the development of antibodies against a biologic?
A: The article under ref. No. 209 in the original manuscript (ref. No. 249 in the revised version) reports that glycation of a recombinant mAb produced by CHO-cells could be substantially (5 times) suppressed by lowering the glucose concentration in the bioreactors. However, neither the high nor the low glycated mAb were tested for immunogenicity. To the best of our knowledge, nobody has tried so far to inhibit AGEs formation in order to prevent Abs development against a biologic. The aim of the present review is to provoke such investigations.

Q: Page 9, line 16: If glycation makes a protein immunogenic, is there evidence that diabetics have antibodies against AGE? Discuss.
A: In the original manuscript we referred to two studies demonstrating that diabetics have antibodies against AGEs (ref. Nos. 133 and 134). Actually, not only diabetics but also healthy individuals develop antibodies against AGEs. While some studies show difference in the titer of plasma anti-AGEs Abs between healthy subjects and diabetics, others fail to find such a difference. Paradoxically, in two studies, we now cite in the revised manuscript (ref. Nos. 168 and 169), the titer of the anti-AGEs Abs was found to be significantly lower in diabetics than in normal controls. To explain this result the authors suggested that tissue-bound AGEs capture plasma anti-AGEs Abs thus lowering the titer of the latter but this suggestion needs further experimental support. This discussion

is now included in the revised manuscript on page 15, lines 17-24.

Q: Page 10 1st paragraph: should be referenced.
A: The text in this paragraph is now referenced (page 12, lines 1-6, ref. Nos. 103-114, 78).

Q: Page 11, line 12: Define SR. Write these in the conventional format e.g. SR-A1 or SR-B1 etc. Which SR do authors mean with respect to CD36?
A: SR Definition: *Scavenger receptors are cell surface receptors that typically bind multiple ligands and promote the removal of non-self or altered self-targets. They often function by mechanisms that include endocytosis, phagocytosis, adhesion, and signaling that ultimately lead to the elimination of degraded or harmful substances* (according to Prabhudas *et al*., *J Immunol* 2014; **192:** 1997). The SRs are written now in the conventional format. Bearing in mind, however, that the SR-nomenclature has been coined quite recently (2014) we left in parentheses the SRs' alternative names as they appear in the original publications. Thank to this question we expanded the information on AGEs-binding receptors by including new references (ref. Nos. 125,126,128,129,131,132,134,135 in the revised version). The revised text is on page 13, lines 11-16. The abbreviation for CD36 according to the new nomenclature is SR-B2. Also, some alternative CD36 names are SCARB3 and PAS4 (see the above cited article).

Q: Page 11, line 13: A bit misleading without saying that scavenger receptors also bind others like lipoproteins e.g. SR-B1 is a receptor for HDL
A: The main focus of the manuscript is on the link between glycation and immunogenicity of protein therapeutics. To be concise and consistent, we consider it irrelevant to go in detail explaining what scavenger receptors are and what they bind. This time, however, we included two references (ref. Nos. 128 and 129), where the reader can find information on that topic. Also, to avoid misunderstanding, we omitted "mainly'' from the sentence on page 11, line 13 (now page 13, lines 16-18): "These receptors are involved mainly in detoxification of AGEs by intracellular degradation (endocytosis)" now sounds "These receptors are involved in detoxification of AGEs by intracellular degradation (endocytosis)".

Q: Page 11, line 26: Apart from AGES
A: "Apart AGEs" is now changed to "Apart from AGEs" (page 13, line 2 from bottom of the revised manuscript).

Q: Page 12 line 13 do you mead Apart from cellular immune responses?
A: Yes, we meant "Apart from cellular immune responses" and the text on page 12, line 13 (now page 14, line 9 from bottom) has been accordingly edited.

Q: Page 12, line 28; change fond to found
A: On the indicated page and line "fond" was corrected to "found'' (now page 15, line 14).
Q: Page 13, line 11: What about anti-AGE-LDL that was formed preventing oxidised LDL from activating macrophages and foam cells?
A: This is an interesting question and in this regard it should be outlined that the anti-atherogenic effect of the anti-AGEs-LDL Abs is disputable yet in so far as anti-AGEs-LDL Abs containing immune complexes are detected in human sera and shown to be important predictors of carotid intima-medial

thickening in patients with type 1 diabetes. This comment is included on page 16, lines 16-19 of the revised manuscript together with the respective references (ref. Nos. 174-176].

Q: Any chance of the AGE-LDL directly competing with LDL if they hang around for long enough if they are not cleared?

A: A: There is an *ex vivo* study (Klein RL *et al*., *Diabetes* 1995; **44:** 1093), showing that when glycated and native LDL isolated from human plasma are mixed with fibroblasts (expressing only the classical LDL receptor), the rate of receptor-mediated accumulation of the native LDL is greater than that of the glycated LDL. With monocyte-derived macrophages, however, the result is opposite - the uptake of glycated LDL is greater than that of native LDL. In other words, AGEs-LDL behaves like oxLDL, and as well-known both are pro-atheromatous. This discussion is not included in the revised manuscript because it is too specific and falls outside the scope of the current review.

Q: Page 15, line 20: Change brides to bridges

A: "Brides" was changed to "bridges" in the revised manuscript (page 18, line 4 from bottom).

Q: Page 15 line 20- Is there any evidence that aggregation of IFNb or other protein therapeutics are immunogenic?

A: Yes, the link between aggregation and immunogenicity is well-documented for several therapeutic proteins, among them hIFNα-2a, hGH and hIFNβ, and in the original version of the manuscript on page 13, line 4 from bottom we wrote: "Aggregates in protein drugs substantially contribute to immune response in treated patients[23,141-146]." The text is left unchanged in the revised manuscript (page 17, lines 4-5) while references are now renumbered to 23, 180-185.

Q: Comment on antibodies against anti-TNF antibodies in treatment of RA.

A: As with most biologics, some RA patients treated with antagonists of the human tumor necrosis factor (TNF) (infliximab, etanercept and adalimumab) develop anti-drug antibodies (ref. No. 172 in the revised manuscript). The TNF-antagonists are in fact anti-TNF Abs of IgG isotype and in light of the current review two events could be proposed: i) if the Fc regions of the therapeutic anti-TNF Abs are also glycated (anti-TNF-AGEs Abs), they could compete with patients' IgG-AGEs for binding RF in human plasma; and ii) if the patients' antibodies against the glycated biologic are specifically directed against the glycated moiety (AGEs) of the anti-TNF-AGEs Abs, apart from binding the drug, they would be also capable of interacting with patients' IgG-AGEs. The net result of this hypothetical and intertwined scenario is difficult to predict, but it should be taken into consideration by pharmacists, pharmacologists and clinicians. This comment can be found on page 16, lines 2-12 of the revised manuscript.

Q: Page 17, line 2: write 'Whether there are any differences…'

A: "Is there any difference" on page 17, line 2 was changed to "whether there are any differences" (now on page 20, line 9) as suggested by the referee.

Q: Page 18, line line: change and to an

A: "and" on page 18, line 9 of the original manuscript was changed to "an" on page 21, line 16 of the revised manuscript.

Q: Page 18, line 10: Has anyone shown increased aggregation of this IFNb to support your idea?
A: Yes, increased aggregation of IFNβ1b (*E. coli*) as compared to IFNβ1a (CHO-cells) is shown by Runkel *et al.* (1998) and is attributed to the lack of a native (enzymatic) glycosylation of IFNβ1b. This new reference (ref. No. 229) is now added to the text on page 21, line 16-17 of the revised manuscript "…and is prone to non-covalent aggregation [229]".

Q: Page 24-27; Both of these have already been defined
A: Ref. Nos. 34 and 35 on page 24 of the original manuscript referred to the same article. Now, we deleted the redundant reference, and left only one in the revised manuscript (ref. No. 34 on page 28).

BAISHIDENG PUBLISHING GROUP INC
8226 Regency Drive, Pleasanton, CA 94588, USA
Telephone: +1-925-223-8242 Fax: +1-925-223-8243
E-mail: bpgoffice@wjgnet.com http://www.wjgnet.com

RESPONSES TO REVIEWER'S COMMENTS (00503083)

There are no questions asked by this referee.

EDITOR'S QUERIES

Audio Core Tip
Q: In order to attract readers to read your full-text article, we request that the first author make an audio file describing your final core tip. This audio file will be published online, along with your article. Please submit audio files according to the following specifications:
Acceptable file formats: .mp3, .wav, or .aiff
Maximum file size: 10 MB
To achieve the best quality, when saving audio files as an mp3, use a setting of 256 kbps or higher for stereo or 128 kbps or higher for mono. Sampling rate should be either 44.1 kHz or 48 kHz. Bit rate should be either 16 or 24 bit. To avoid audible clipping noise, please make sure that audio levels do not exceed 0 dBFS.
A: Audio Core Tip is provided as required.

Q: Please add PubMed citation numbers and DOI citation to the reference list and list all authors. Please revise throughout. The author should provide the first page of the paper without PMID and DOI.
PMID (http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=PubMed)
DOI (http://www.crossref.org/SimpleTextQuery/) (Please begin with DOI: 10.)**
A: Where available, PubMed citation numbers and DOI are added to the reference list and all authors are listed for each reference. To ref. Nos. 26, 86, 116, 117 and 208 we provide Internet links.

Q: Please provide the decomposable figure of Figures, whose parts are movable and can be edited. So please put the original picture as word or ppt or excel format so that I can edit them easily.
A: Figures are provided in Word and Excel formats.