Analysis of BAFF-dependent signatures and biology in the intestinal mucosa of patients with inflammatory bowel disease

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Protocol responsible:

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Background

BAFF, a member of the TNF Superfamily, is a key player in B cell biology (Mackay F. et al. Nature Reviews Immunology, 2002). Its functions include promotion of B cell survival and activation of class switch recombination. But the effect of BAFF on immunity is not limited to B cells it is also an important co-stimulator of T cells as well as activator of monocytes and APCs.

Its role in different inflammatory disorders, SLE, RA has been reported (Vincent FB. et al. Nat Rev Reumathol, 2014; Wei F. et al. Cytokine, 2015) as well as the effectiveness of a-BAFF antibody (Belimumab) in these diseases. In IBD one new study report its potential involvement in the pathogenesis (Zhang P. et al. Dig Dis Sci 2016).

We believe that BAFF could be contributing to IBD pathogenesis and investigation is needed to explore this TNF superfamily member and its establishment as a potential therapeutic target for IBD.

Objective

The overall objective of this proposal is to set the stage for further investigation of anti-BAFF antibodies for the treatment of inflammatory bowel disease (IBD).

To do that we propose to obtain data on the expression of BAFF and BAFF-related genes and proteins in the involved mucosa of IBD patients (both ulcerative colitis (UC) and Crohn's disease(CD)).

Moreover, the biological effects of anti-BAFF blocking antibodies will be tested using human colonic explants from IBD patients (with active disease).

Overall Strategy

Given the lack of evidence for a role of BAFF signaling in IBD, our approach will consist of different stages. **The first stage (WP1 and WP2)** is intended to provide expression data using whole biopsy transcriptomic signatures available to our group from a cohort of IBD and control individuals. By using correlation analysis of all transcripts measured for each biopsy, this first approach will allow us to identify potential sources and target cells/pathways regulated by BAFF in the context of IBD. The data generated from this analysis will be validated in a second independent longitudinal cohort of IBD patients starting treatment with biologics.

A second stage (WP3) will test the direct impact of blocking BAFF on human tissue explants by measuring selected target genes identified in the first stage of the project. While we believe that these experiments can be extremely informative, we also foresee the possibility that the effect of anti-BAFF may require longer analysis times than those for explant cultures. We recognize this possibility and propose a short pilot experiment that will test a reduced number of patients (n=5) who present active inflammation. A potential extension of this study (not included in this proposal) will be discussed and carried out if the results of these first samples demonstrate biologic effects of anti-BAFF on tissue cultures.

Finally, a **third stage (WP4)** will develop spatial transcriptomics approaches for tissue sections from IBD patients to reveal the tissue localization of BAFF, BAFF-related (APRIL, BAFF and APRIL) receptors, and BAFF-dependent signatures (to be determined during the first stage of the project). Since we would perform tissue RNAseq localized approaches to a close-to-single cell resolution, we would be able to relate and explore contact-dependent and location-specific BAFF signals in the context of intestinal inflammation.

Work plan

All the sample and data collection, performance of experiments and analysis and interpretation of data will be held by the research team of Dr. Azucena Salas at IDIBAPS. GSK will provide the a-BAFF antibody and isotype control needed for WP3 and will be crucial in the interpretation

of data given their expertise in the protein BAFF and mechanism of action in other diseases.

First stage:

- **WP1**. Exploring whole-genome transcriptional data for BAFF-correlated genes in human intestinal biopsies.

  Microarray (Affymetrix GeneChio Human Genome U133 Plus 2.0) data from 77 human intestinal samples (17 healthy colon, 15 UC, 23 ileal CD and 22 colonic CD) is available in our laboratory (unpublished). All patient samples (UC and CD) were obtained from actively inflamed (endoscopically active) mucosa. “Bioinformatic analysis of relevant biological processes and pathways from available datasets of selected subpopulations of Crohn’s disease (CD) patients and healthy volunteers”. Registro: 2010/6167.

  A correlation analysis of BAFF (*TNFSF13B)* expression with all mapped genes (over 20.000 genes) will be performed to potentially identify both main BAFF-producing cells in active disease, as well as direct targets of BAFF biology in the intestinal mucosa.

  Deliverable 1 (Month 1): A list of all genes within the microarray and their coefficients of correlation and statistical significance will be delivered. Genes of interest will be selected together with the team at GSK for further analysis in WP2.

  Deliverable 2 (Month 2): A selected list of genes whose expression shows the highest correlation with BAFF throughout the samples will be provided for further validation. Selection of genes will be based not only on the statistical significance of the correlation analysis, but also on the cell subsets/pathways involved to ensure that all potentially biologically relevant genes discovered during these steps are included in subsequent analysis.

- **WP2**. Validation of the BAFF-correlated transcriptional signature in a longitudinal cohort of IBD patients receiving biologics to induce remission.

  Custom-designed TLDA (48 genes per sample format) will be ordered. In addition to the list of genes selected in WP1, we will include 2-4 endogenous controls for the relative expression analysis of target genes.

  A cohort of samples already collected in our unit will be used for validation. “Biologic Assessment of Response and relation to Clinical activity, Endoscopic and radiologic Lesions, in IBD patients ON Anti TNF therapies (BARCELONA) V.1 de 25 d’Octubre de 2012”. Registro: 2012/7956.

  Samples were taken from patients starting treatment or switching biologics (anti-TNF, vedolizumab or ustekinumab) in our IBD Unit. Biopsies are available at baseline (week 0), end of induction (week 12-14) and at week 46.

  Total RNA will be isolated from these biopsies and 500 ng will be retro-transcribed and analyzed for RNA expression using the above-described TLDAs (48 genes/sample).

Table 1. Number of samples available for transcriptional analysis in our laboratory. Patients receiving either anti-TNF, vedolizumab or ustekinumab underwent endoscopic evaluation and sample collection at week 0, week 14 and week 46. Samples colored in gray indicate those used for Deliverable 3; those in pink for Deliverable 4 and those in blue for Deliverable 5.

	Week 0	Week 14	Week 46
		Anti-TNF*	
UC	34	34	25
cCD	30	28	25
iCD	29	29	22
		vedolizumab	
UC	18	14	10
cCD	2	2	1
iCD	5	3	2
		ustekinumab	
UC	1	-	-
cCD	8	5	1
iCD	14	8	3

Deliverable 3 (Month 6): Relative expression of all selected genes in a cohort of well-characterized patients. A total of 87 UC samples, 68 colonic CD and 77 ileal CD samples (Table 1. Samples in grey). The correlation between BAFF expression and all other genes analyzed by Taqman assay will be provided to confirm data in WP1 using an independent cohort.

Deliverable 4 (Month 8): Analysis of samples collected at week 0 (before starting biologic treatment; Table 1 samples in pink quadrant: 53 samples from UC, 30 samples from colonic CD, and 48 ileal CD samples) will be performed separately to identify patterns of BAFF and BAFF-dependent genes within subsets of well-characterized active patients.

The effects of demographic, clinical and endoscopic variables on gene expression will be assessed to determine the influence of these variables on patterns of BAFF (and BAFF-correlated genes) expression.

Deliverable 5 (Month 9): Analysis of patients receiving anti-TNF treatment (week 0 and week 14 samples) will be reported here. The effects of anti-TNF treatment on BAFF and BAFF-dependent genes will be assessed in a cohort of UC and CD patients (Table 1; in blue quadrant). This cohorts includes responders and non-responders based on endoscopic findings at the end of induction (week 14).

Second stage:

- **WP3**. Directly test the effect of anti-BAFF on biopsy explant (n=5 active UC or CD samples). Collect RNA and sups after o.n. (16 h) culture of biopsies with anti-BAFF or an isotype control. Measure target genes on different cell types by PCR analysis. Include anti-TNFα as control if enough biopsies are available.

  Deliverable 6 (Month 9): A first analysis of selected BAFF (or TNFα) target genes will be reported in tissue explants from 5 patients treated with vehicle control (Ig), anti-BAFF antibody or (whenever possible) infliximab. Expression of up to 12 genes will be tested in these group of 10-15 samples.

Third stage:

- **WP4**. Analysis of spatial transcriptomics in patient intestinal tissues using spatial RNAseq analysis. The objective of this WP is to provide information on the location of genetic signatures within the inflamed mucosa of patients with active IBD. We will specifically focus on providing the locations of BAFF and all BAFF-related genes identified and potentially validated in the previous WPs. This methodology allows visualization within the tissue and quantitative analysis of the transcriptome of IBD intestinal mucosal sections.

  Deliverable 7 (Month 12): Spatial transcriptomics using 10XGenomics recently acquired technology will be used. We will use histological sections of patient intestinal mucosa samples on glass slides with arrayed oligonucleotides containing positional barcodes. High quality cDNA libraries with precise positional information for RNA-seq will be generated for a total of 6 samples.

Patients

We will need to recruit patients for the obtention of new samples to be used in WP3 and WP4.

WP3. 5 CD or UC active patients undergoing routinary endoscopic evaluation at Hospital Clínic de Barcelona will be selected. Patients will be informed and will sign the general informed consent form "CI_col.lecció_MII_cat/cast_v3 01062018" used in the IBD Unit of the Hospital Clínic de Barcelona. Endoscopy is performed by an experienced gastroenterologist of the IBD Unit and biopsies will be harvested in sufficient number to perform the experiment (minimum of 2, 3 whenever it is possible) based on her medical criteria.

WP4. Similarly to WP3, 6 patients with active IBD (ideally 3 CD and 3 UC patients) undergoing routinary endoscopic evaluation will be recruited. In this case, only 1 biopsy per patient will be enough.

For both WP, we mentioned the number of patients to be reported to GSK. However we anticipate that we will need to optimize/troubleshoot both proposed experiments. For this reason we foresee to recruit more patients for each work package. We plan to include 10 CD or UC active patients for WP3 and 10 more for WP4.

Patients' inclusion criteria

Patients males and females aged 18 years of age or older manifesting a diagnosed IBD (for at least 3 months prior to screening) with active inflammation (UC patients with a Mayo Endoscopic score of ≥2 and CD patients with a SES-CD score ≥7)

Patients' exclusion criteria

1) A diagnosis for an active serious infection, including localized infections
2) Any condition or disorder that, in the opinion of the investigator, might confound the study results or pose additional risk to the subject
3) Any contraindications for one or more biopsy collection

Statistical Analysis

The correlation analysis of BAFF (*TNFSF13B)* expression with all mapped genes will be performed using a Pearson test to obtain their coefficients of correlation and statistical significance.

RNA expression (microarray or RNA-Seq data) in all different groups will be analyzed using R statistics tool. Differential expression analysis will be performed using limma and edgeR

packages. To correct for multiple testing, the false discovery rate (FDR) correction will be applied. A gene will be considered differentially expressed when significant at 5% FDR and having a fold-change higher than 1.5.

Analysis of mRNA expression (Real Time PCR) and/or cytokine production in ex vivo explants will be performed using a Mann Whitney or Kruskal Walllis non-parametric test with an FDR correction for multiple comparisons when needed.

Ethical and legal statements

The protocol will be performed following the guidance of the Declaration of Helsinki (64th WMA General Assembly, Fortaleza, Brasil, October 2013) for the ethical principles for medical research involving human subjects.

The protocol will be performed in accordance with the legal requirements of the Ley 14/2007 de 3 de julio, for biomedical investigation.

Patients will be thoroughly informed and will sign the general informed consent form "CI_col.lecció_MII_cat/cast_v3 01062018" used in the IBD Unit of the Hospital Clínic de Barcelona.

Data processing and archiving of records. Data confidentiality.

In this protocol we use data associated with biological samples already collected in previous protocols (WP1 and WP2). In this case the associated data source is the SAP software of Hospital Clínic de Barcelona and our own databases:

- Located in the server of Hospital Clinic of Barcelona (Unit F :).
- Access control: restricted to authorized research personnel only (Azucena Salas)
- Risk management: policy guidelines for data protection at the Hospital Clínic de Barcelona (HCB) are followed.
- In the Patient Informed Consent (CI) we state the compliance with Regulation (EU) 2016/679 on the protection of individuals with regard to the processing of personal data and the free circulation of such data.

New data will also be registered associated to the collection of new biological samples for WP3 and WP4. The purpose of this data collection is to have the information necessary for the correct classification of patients and samples and the interpretation of the data obtained after analysis of the samples. For the collection, storage and accessibility of new data the same criteria as for archived data mentioned above apply.

Management, communication and transfer of personal data of all participants will comply with the EU Regulation 2016/679 of the European Parliament and of the Council of 27 April 2016 on the protection of individuals with regard the management of personal data and the free circulation of data, being mandatory from May 25, 2018 and the Organic Law 3/2018, of 5 December on personal data Protection and guarantee of digital rights. The legal basis justifying the data management is the signed consent of the patient, as stated in Article 9 of EU Regulation 2016/679.

All samples and associated data will be subjected to a coding process. Any personal data will become part of the File Research, for which HCB is responsible. Only authorized personnel of HCB can correlate your identity with the aforementioned codes. Even when the results of the conducted research are published in scientific circles, your identity will never be revealed. The identity of participants will not be available to any other person except for a medical emergency or legal requirement.

Health authorities, the Ethics Committee for Research and personnel authorized by the study sponsor will have access to identifiable personal information only when necessary to check data

and study procedures and always maintaining confidentiality in accordance with current legislation.

Only the coded data will be transferred to third parties or other countries and in any case will contain information that can identify the participant directly (such as name, initials, address, social security number, etc.). In the event that this transfer occurred, it would be for the same purpose of the study described and ensuring confidentiality.

If there was a coded data transfer outside the EU, whether in entities related to the hospital where the patient participates, service providers or researchers who collaborate with us, participant data will be protected by safeguards as contracts or other mechanisms established by the data protection authorities.

As promoters of the project we commit to treat the data according to EU Regulation 2016/679 and therefore to keep track of the data management that we carry out and at the same time to conduct a risk assessment of the management to know what steps we need to apply and how to do it.

In addition to the rights already considered by the previous legislation (access, modification, opposition and cancellation of data, deletion in the new regulation) now participants can also limit the management of the data collected for the project in the case to be incorrect, request a copy or transference to a third party (portability). To exercise these rights, individuals should address to the principal investigator of the study or the Data Protection Officer of the Hospital Clínic of Barcelona through the email protecciodades@clinic.cat. The patient has also the right to file a complaint with the Catalan Agency for Data Protection in case that HCB carries out any action that infringes upon his/her rights.

Data can not be deleted if a patient leaves the study to ensure the validity of the investigation and comply with legal obligations and requirements for authorization of medicinal products.

The investigator and the sponsor are required to retain the data collected for the study at least up to 5 years after completion of the project. Subsequently, the personal information will only be retained by the center for health care and other promoter's scientific research in the case patient had given his consent, and if permitted by law and ethical requirements.

Funding

This protocol is funded by GlaxoSmithKline Pharmaceutical Company. Financial budget is presented attached to this protocol.

Publication Policy

IDIBAPS and GSK agree to publish the results of this Project in scientific circles relevant to the research field.