Abstract: The purpose of this research proposal is to suggest the possibility that the three conserved regions of the Receptor binding domain of HCV (the HVRI, HVRII and IgVR) are the epitopes of HCV where the virus binds to SR-B1 and CD81. That though fragmentation of the RBD at both the HVRII and amino acids in IgVR, to separate amino acids 613-618 from the RBD in E2 glycoprotein, to use in a functional protein microarray against CD81 assays. This will hopefully show neutralization of HCV in vitro. Then a further test of thee fragments in Trimeria mice will further prove neutralization of HCV by preventing HCV infection in the mice. Thus making it possible for development of a recombinant vaccine for HCV.

Keywords: HCV, Recombinant vaccine, CD81, HVRII, IgVR, epitopes

1. Introduction

This paper will try to prove that the amino acid residues found in the journal references below can be used to identify the epitopes in the E1/E2 glycoprotein region of HCV so that the papers information can be used to create a recombinant vaccine for Hepatitis type C. The first part of this paper is to state the effects of Hepatitis type C. This starts by saying that Hepatitis type C is a vicious liver disease that effects phase one and two liver functions in the people who it infects. That in this medical site [1], while not describing the effects of HCV on liver function it tries to explain liver detoxification of substances that enter the liver. That in phase 1, fragments enter the liver and are reduced in size which strongly suggest the functions of the SR-B1 receptor [2] and thus a scavenger receptor [3], because of its description, it has the ability to handle high density lipoproteins and thus can break down high density lipoproteins for Phase 2 liver function to proceed. That in Phase 2 liver function the elements of Cysteine, glycine and sulfur is added to the fragments already processed by Phase 1 liver function so that they are less toxic so they can be secreted as bile [1]. This paper will suggest that the HCV virus causes so much liver damage by effecting the ability of the liver to break down toxins in Phase 1 and Phase 2 of liver function by not processing high density lipoproteins properly as well as causing cholesterol trafficking problems [4]. Showing that while NPC1 gene is not the binding factors in a Hepatitis type C infection it does still effect the cholesterol trafficking factors that the NPC1 gene controls. The Hepatitis type C virus is composed of three structural proteins. The core protein, the E1 glycoprotein and the E2 glycoprotein. Then the non situational proteins of Hepatitis type C are the p7 viroprin, the glycoprotein. Then the non situational proteins of Hepatitis type C virus is composed of three structural proteins. The NS3-4a (a complex protease protein and a NTPase/RNA that controls RNA helicase activities, the NS4B and NS5A proteins and the NS5B which is a RNA-dependant RNA polymerase [5] On the virus membrane the glycoprotein E1/E2 is the viruses receptor binding site [6]. The genes associated with E1/E2 are CD81, SR-B1, Occuldin and CDLN1 [7]. I suggest that the OCLN gene pairs up with CD81 because of how many regions CD81 has (N and C terminus, 4 transmembranes, SEL and LEL) [8][9][10] and thus I suggest these 8 domains coincide with the 9 domains of the OCLN [8] gene where as the LEL pairs up with extracellular loop 1, the SEL with intercellular loop 1, each 4 transmembrane domains and or regions pair up with transmembrane domains and the N and C terminus pairs up with the N-terminus and C terminus domains [8]. This journal [11] also suggest that there is several discontinuous CD81 binding motifs with in E2 that makes folds including the polyprotein residues Trp420, Trp437, Leu438, Lue441, Phe442, Tyr527, Trp529, Gly530 and Asp532. That these are the names of the sections of E2 that coincide with CD81 and thus also line up with the sections of Occuldin. These are the tight junctions between CD81 and CDLN1. There are 2 hypervariable regions and a Intergenotypic region of the E2 glycoprotein [12] that contain the 3 domains to E2 tertiary conformational epitope structure [13]. This thus suggest that E2 has three epitopes in its within HVRI, HVRII and IgVR. Within this glycoprotein receptor binding site there is an independent folding domain that coincides with hyper variable region 1 and 2. That spans polypeptide residues 384 to 661. These residues can be effectively expressed by their secretion from cells while maintaining the binding of genes CD81 and SR-B1 [6][11]. This thus suggest that tetraspain CD81 and scavenger receptor class B (SR-B1) are HCV cellular entry factor, with the tight junctions of CDLN1 creating a seal between the domains of Occuldin that create the structural stability of CDLN. Occuldin’s 8 domains (N-terminus domain, Tran membrane domain 1, extra cellular loop 1, Tran membrane domain 2, intercellular loop, Tran membrane domain 3, extra cellular domain 2, Tran membrane domain 4, and c-terminus domain) create the tight junctions of CD81 and CD81s composed of 236 amino acids that coincide and interlock with the 8 domains of Occuldin suggesting that they coincide the polyphonies stated above (Trp420, Trp437, Leu438, Lue441, Phe442, Tyr527, Trp527, Gly530 and Asp532) by connecting CD81 with Occuldin by the tight junctions of CDLN1 [14][15]. Within the receptor binding domain (RBD) are three discrete variable regions that are separated into the two Hypervariable regions and one Intergenotypic region [11]. The N-terminus of E2 lies in Hypervariable region 1 and has 11 glycosylation sites [11]. It is the binding site for SR-B1 because of its content of
glycans. This is where E2 binds to SR-B1 by way of a glycan. That SR-B1 binds to high density lipoproteins [2] and while E2 is not a lipid it does contain glycans, just as glycolipids do thus binding to SR-B1 like a glycolipid does [16]. The amino acid residues that this domain of E2 binds to SR-B1 by is amino acid residues 384-410 [11]. The final two regions lie in Hypervariable region 2 [11]. This epitope in region 2 (epitope 2) part of HVR2 could be part of the extracellular loop region of Occuldin and thus part of CD81 and its extracellular loop (LEL) [10],[17],[18]. This binding site binds to amino acid residues 474-482 and then the final region of E2 at the end of Hypervariable region 2 that binds to CD81 is part of the intergenotypic variable region of E2 and binds to amino acid residues 571 to 580 [11]. Both the IgVR and HVR2 are flanked by Cysteine residues on each side of them, separately, and all domains (the 3 variable regions) are suggested to be solvent exposed and not part of the core domain [11]. I suggest the viral entry sequences of amino acids 412 to 423 are part of Hypervariable region 1 that contains the sequence that SR-B1 binds to but is not part of E2 folding domain. HVR2 and the IgVR isn't either but because it has flanked Cysteine residues it separates the amino acid 412-423 region and SR-B1 region from HVR2 as the IgVR is separated from the HVR2 region by disulfide bridges [19],[11]. That alpha interferon treatment is used on the HVR2 region because the HVR2 region is responsible for E1/E2 function [20]. It is shown that when HVR1 and HVR2 is removed from E2 661 that the IgVR compromises binding of CD81 50% of the time thus the IgVR is important to the structural design of the E2 RBD so as to allow binding. This suggest the IgVR connects the HVR1 and HVR2 regions to the core of E2 and thus to the rest of the virus. That if one could remove the IgVR the virus couldn't bind to CD81. Though because it has a glycan at amino acid 576 a antibody cant appropriately bind to this region just as a antibody cannot bind to the HVR1 region as well because of its 11 glycosylation sites. Another study shows that removal of the HVR1 region also increased binding of E2 to CD81, this also suggest the HVR2 is more likely to bind to CD81 where as HVR1 may be more likely to bind to SR-B1 [12]. The study [20] also suggest that the amino acids in the IgVR region (613-618) are also responsible for the binding of IgVR to CD81 thus suggesting that HVR2 and the IgVR bind to CD81 where as HVR1 does not. If the virus first attaches to CD81 and SR-B1 because of its glycan then it can bind both to HVR1 and SR-B1 but if you were to stop binding to the HVR2 and the IgVR then the glycans in the HVR1 region couldn’t bind. Plus if you block amino acids 613-618 in the IgVR as well the virus could be neutralized. This means to prevent HCV infection and take care of the problem we may have to set aside all of HVR1 and focus on HVR2 and aa 613-618 as the main epitopes of CD81 interaction thus the main the antibody target site that stops entry into the host receptors by just blocking virus entry into CD81.

2. Hypothesis

If these three conserved regions in the RBD are the epitopes of HCV then HVR2 and amino acids 613-618 in the IgVR region can be used as a template for fragmentation to make a recombinant vaccine. First the core E2 glycoprotein (gp70)[21] has to be fragmented by using the disulfide bridges flanking the HVR2 region as a restriction site to remove the HVR2 (containing amino acids 571-580) for a recombinant vaccine. This can be done by using the reductant tris(2-carboxyethyl)phosphine (TCEP) to fragment HVR2 at the disulfide bridges and separate it from E2 [22]. Then in the IgVR region use the amino acids 609 and amino acid 623, which are amino acid Leucine, as restrictions sites for restriction enzyme PcsI, XcmI or Leucyl Aminopeptidase [23],[24],[25] to fragment a section with amino acids 613-618 in it, for use in the same recombinant vaccine. After you do this take both of the these new fragments and use them in PCR to replicate them for invitro testing of neutralization of the HCV virus. Then move on to the injection these fragments into Trimeria mice models to see if the same results from the in vitro test occur in the mice and thus the mice having no infection of HCV. The HVR1 region can't be used because it is glycosylated and thus a amino acid sequence can't be used to bind to this region though because a glycan can bind to SR-B1 (even though it suggest the HVR1 is one of the epitopes that binds to SR-B1 and or is part of the overall epitope of E2). The other epitope or part of the epitope is the IgVR region but is not accessible either because of its glycan at amino acid 576 but its 613-618 region could be used as a binding epitope to CD81. The last epitope or region of the E2 RBD that is accessible is HVR2 thus the main candidate for the creation of a recombinant vaccine. The below stated methodology will have to be conducted by a supervising virologist in a lab who has the proper funding and resources to conduct the experiment.

3. Results and Discussion

The main questions in the research proposal are to identify the three conserved regions (HVR1, HVR2 and the IgVR) they are the regions of the epitopes of HCV with the HVR2 and aa 613-618 in the IgVR region being the target area for vaccine development in which can be tested by the methodology below and whether a test, in vitro and in mice, of fragments of these sections taken from the RBD, will neutralize HCV and if they do a possible recombinant vaccine can be developed for use in humans.

4. Methodology

First acquire HCV and CD81 assays from a company and or companies that sell these assays and inject Trimeria mice with HCV. Then use mass spectrometry, ion chromatography or edmund degradation to identify the RBD stated in journal reference 11 [11] so that you can identify the three conserved regions stated to be in the RBD (the HVR1, HRV2 and IgVR). From this use the region previous to fragment a section with amino acids 613-618 out of the RBD or Leucyl Aminopeptidase will have to be conducted by a supervising virologist in a lab who has the proper funding and resources to conduct the experiment.

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Aminopeptidase at amino acids 609 and 623 in the remaining section to remove a section 613-618 for use in a mass spectrometry, gel electrophoresis or ion chromatography to sort the HVR2 fragments and the amino acid 609-623 fragments from the restriction enzyme digest of E2 glycoprotein (gp70) then use PCR along with these fragments to make a recombinant vaccine. From these amplified fragments (PCR) one would then do a functional protein microarray to see if they bind to a CD81 assay. If they bind then it shows neutralization, invivo, might be possible. Then a test can be done on Trimera mice models described in this journal [26] to test the viral infection rate after these fragments of the recombinant vaccine when it is injected. Other ways to test this hypothesis is to use Computational analysis procedures like Structural Alignment and protein structure prediction to see where the Receptor binding domain folds and then a protein sequence alignment procedure like BLAST to test where within those folding sequence does CD81 bind to the virus. This whole experimental process will have to be conducted by a certified virologist who has a lab with the equipment stated above and serve as the supervising scientist on the project as I do not have the resources, funding, certification or experience to conduct the experiment. This thus makes a time table for the experiment and collection of data unknown. The basis for my Hypothesis comes from self study of the internet of medical sites, medical journals and of other sources like Wikipedia to get an idea of the mechanisms of HCV and its effects on the liver then contemplation and analysis of what was read.

5. Results and Set Backs

If the experiment is successful and the fragments created from the restriction and redundant process bind to HCV and stops viral infection then a recombinant vaccine for Hepatitis Type C can be developed and clinical trails can be conducted after the final experiment with the Trimera mouse model. The setbacks are that the restriction sites may not be disulfide bridges and that HR2 and the IgVR (especially before and after aa 613-618 of the IgVR) are not the epitopes thus the restriction sites may be another sequence that would have to be identified by mass spectrometry, ion chromatography or edmund degradation of the RB2 to see what the proper epitopes are. Another setback would be having to identify any proteins of significance to proper cell function that CD81 is a receptor for and that uses the regions on HVR2 and the IgVR that are the target sites as entry mechanism into CD81 thus making a recombinant vaccine that uses these segments potentially harmful for a person. The other set back is Im disabled and my only source of income is Social security income and with my level of factual education (GED with some college but not accredited) I could not be hired by any institution for this level of work or research nor could I ever get paid enough at a minimum wage job to acquire what I needed for a lab. Getting a grant which would affect my SSI income. I could run a crowd funding campaign in which to raise funds to hire a certified virologist to conduct the experiment where a paper could be written and published thus that paper could be presented to a biopharmaceutical company so they could decide to do clinical trials on the data collected. Though crowdfunding campaigns are not reliable. Which if I had some proof this hypothesis had merit then I could collect the funds though crowd funding to continue the process to see if this experiment works, publish a paper and then present it to people who could do clinical trials so as a vaccine could be created. The other possibility is to skip the crowd funding and have a institution or company use this research proposal and maybe give me other opportunities as well so that I may continue research on other disease.

6. Research Location

At this time there is no research location identified and can’t be identified till after a crowd funding campaign is done, grant for funding is received and or a virologist, though institution or independently, who has the proper resources and a lab are hired or takes on the project.

7. Time table for experiment

Once the crowd funding campaign is finished or a grant is received it is according to that virologist that is hired to decide on what a proper time table is for the experiment plus whatever additional time it takes to test the level of viral infection of the Trimera mouse model and the time to collaborate with other scientist who have trimera mouse models to show that the CD81 fragment can be used for a recombinant vaccine in clinical trail.

8. Significance of Research

HCV causes liver damage and death to ones who are infected with the disease and if infection can be prevented then there would be no need for other therapies to fight HCV as well as eventual eradication of the disease. That this experiment may prove a way to make are recombinant vaccine that may someday eradicate HCV ad make infection impossible.

References

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Author Profile

Jonas Tyler Doran, 37 year old male from Barberton Ohio, United states of America. I have Schizoaffective disorder and am on the federal welfare system. I went to college briefly (unaccredited) for psychology and physics. I got into other disciplines of science on his own alter that stopped going to college. With this paper I hope to fulfill one of my dreams of finding a way to make a vaccine for HCV. In my past I have experienced people I know who have used drugs intravenously and maybe if someone they knew helped vaccinate them from HCV (a disease that effects this type of drug user) maybe they would stop using the drug. Give them hope in a sense. I also hope to get industry interested in the concept for testing and development so that the world can have a vaccine for the masses. This article has been posted on my blog site and Wordpress.com, on Linkedin and on Facebook but till this point in time, hasn’t been published in any journals. The only competing interest or regulations to this concept would be the opinions of Community Support Services in Akron Ohio, The federal Social Security Administration, Jobs and Family services of Summit county and Akron Metropolitan Housing Authority of Summit county. Though these are more of finical restrictions than restrictions of publishing content or research.