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The essential microenvironmental role of oligomannoses inserted into the antigen-binding sites of lymphoma cells.

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The essential microenvironmental role of oligomannoses specifically inserted into the antigen-binding sites of lymphoma cells.

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Abstract

There are two mandatory features added sequentially *en route* to classical follicular lymphoma (FL): first the t(14;18) translocation which upregulates BCL2; second the introduction of sequence motifs into the antigen-binding sites of the B-cell receptor (BCR), where oligomannose-type glycan is added. Further processing of the glycan is blocked by complementarity-determining-region (CDR)-specific steric hindrance, leading to exposure of mannosylated Ig to the microenvironment. This allows interaction with the local lectin, DC-SIGN, expressed by tissue macrophages and follicular dendritic cells. The major function of DC-SIGN is to engage pathogens, but this is subverted by FL cells. DC-SIGN induces tumor-specific low-level BCR signaling in FL cells and promotes membrane changes with increased adhesion to VCAM-1 via proximal kinases and actin regulators , but, in contrast to engagement by anti-Ig, avoids endocytosis and apoptosis. These interactions appear mandatory for early development of FL prior to acquisition of other accelerating mutations. BCR-associated mannosylation can be found in a subset of germinal-center B-cell-like DLBCL (GCB-DLBCL) with t(14;18), tracking those cases back to FL. This category was associated with more aggressive behavior, and both FL and transformed cases, and potentially a significant number of cases of Burkitt's lymphoma which also have sites for N-glycan addition, could benefit from antibody-mediated blockade of the interaction with DC-SIGN.

Introduction

Establishment of classic follicular lymphoma (FL) involves multiple B-cell opportunistic changes, beginning in the bone marrow where the t(14;18)(q32;q21) translocation upregulates anti-apoptotic BCL2. The resulting progenitor cells can undergo the normal processes of antigen engagement and differentiation in the germinal center (GC) and may remain harmless. However, other events can occur which allow the cells to survive and proliferate in the hostile GC, where the stay of normal B cells is only transient. The strategy here is to harness and modify interactions with the microenvironment. Since the B-cell receptor (BCR) is a key sensor of the microenvironment, it is not surprising that this offers a major opportunity for subversion. BCR-based subversion varies with the nature, behavior and location of B-cell tumors, and in some cases, such as chronic lymphocytic leukemia, clearly involves responding to autoantigen.[1] FL, a GC-derived tumor, has evolved a different strategy.

The structural peculiarity of the BCR of classical FL cells has been well documented and its mandatory nature reveals a feature required for lymphomagenesis.[2] It arises as a simple modification of the surface immunoglobulin variable region that occurs during somatic hypermutation, introducing sequence motifs (Asn-X-Ser/Thr where X can be almost any amino acid except Pro) then available for addition of glycan. In this way FL cells are capturing a normal mutational mechanism, followed by positive selection of cells with these motifs. The same feature is also seen in some other germinal center tumors, but rarely in normal germinal center B cells or in other B-cell tumors such as chronic lymphocytic leukemia or myeloma.[3] Even more strange is that the introduced glycan terminates prematurely at the high mannose stage, with a range of inserted mannoses from M5-M9[4] (Fig.1a). The halt on further processing is in stark contrast to the Ig constant region N-glycosylation sites in the same Ig molecule which are fully processed to complex sugars.[5] This leads to the odd situation in FL of expressing “naked” high mannoses in the antigen-binding site of the surface Ig receptor, a feature not generally found in cell

surface glycoproteins. The necessity of this peculiar modification of the BCR for FL is underpinned by the fact that surface Ig expression is retained in this lymphoma, even though one allele is lost due to the t(14;18) translocation.

Knowing that the motifs were introduced during somatic hypermutation tells us that they are present at the very early stages of lymphoma development, possibly even in the precursor cells which are detectable in normal individuals.[6] Potentially the presence of motifs could reveal the "committed precursor cell" [7] and motifs have been found in 2/2 cases of FL *in situ.*[8,9] Among the vast inter-patient heterogeneity of FL at the genetic/epigenetic level,[7] the consistent presence of N-glycosylation sites in the BCR stands out as a common feature. The question then arises as to the purpose of the exposed mannoses, and, since sugars generally interact with lectins, which microenvironmental candidate could be interacting with the mannosylated surface Ig (sIg-Mann)?

DC-SIGN (dendritic cell–specific ICAM-3–grabbing nonintegrin) (CD209) was an obvious candidate as it is present in FL tissue[10] and binds to mannoses.[11] The nomenclature of DC-SIGN is misleading as it is not dendritic cell-specific and it binds to other molecules beside ICAM-3. In fact, tetrameric DC-SIGN binds specifically to the Ig-Mann expressed on FL cell surfaces via its carbohydrate recognition domains (CRD) (Fig.1b), and does not bind to normal B cells or other blood cells .[12,13]

This review makes the case for the significance of the *sine qua non* interaction between FL cells and DC-SIGN for lymphoma development/maintenance. It addresses the expression of DC-SIGN *in vivo* and the consequences of this interaction *in vitro* and suggests a therapeutic approach by antibody blockade, potentially at an early stage of disease. For DLBCL, the presence of acquired N-glycosylation sites (AGS) in the Ig complementarity-determining regions (CDR) appears to reveal a subset derived from FL and could add prognostic value.

Localization of DC-SIGN in FL tissue

The expression of IL-4 in FL tissue[14] sets the scene for polarization of macrophages to the M2 type, and explains the expression of DC-SIGN by these cells in FL, located mainly in the interfollicular zone.[10,15] More recently our collaborators have also detected DC-SIGN on follicular dendritic cells (FDC) in FL (Fig.2)[15] (Burack Richard, Zhang Yi, manuscript in preparation). The double staining of macrophages (red CD163) plus DC-SIGN (green CD209) gives a cyan color; that of FDCs (blue CD23) plus DC-SIGN (green CD209) gives yellow (Fig.2) showing that in this illustrative case both macrophages and FDCs express DC- SIGN. The role of FDCs in FL is unclear, although it has been suggested that they may be critical for the early stages of lymphoma development when the network which they form in normal germinal centers (GC) is intact.[16,17] In a normal immune response, FDCs bind immune complexes via Fc receptors or complement receptors.[18,19] These are taken up into cycling endosomes which are then presented to B cells.[20] Perhaps more importantly for FL, FDCs express a range of apoptosis regulators and are able to sense the local microenvironment as well as to interact with normal B GC cells.[19] The ability of FL cells to bind to FDCs via the sIg-Mann-DC-SIGN axis could allow the tumor to hijack a supportive interaction without the need for antigen.

The frequency and distribution of FDCs in FL, among the infiltrating lymphoma cells, is complex and varies between cases. However, where FDCs persist, there appears to be a coordinated expression of CD23 and DC-SIGN, as found by our collaborators, Richard Burack and Yi Zhang (Fig.2). More details of the cellular composition of the FL tissues, and of the expression of DC-SIGN, will be described in an accompanying review by Drs Radtke and Williams. The main conclusion at this point is that FL cells can interact with both FDCs and with M2 macrophages, both expressing DC-SIGN, and that, although cellular composition will vary

between cases, there is always DC-SIGN available in the microenvironment of lymphoma tissues.

BCR Ig mannosylation in lymphoma

Follicular Lymphoma

The high incidence of AGS motifs for glycan addition in the IGHV of classical FL has been widely confirmed. [21,22] In a tumor noted for its heterogeneity, AGS are virtually universal and even the few cases with no sites in the Ig heavy chain variable region (IGHV) could have them in the IGV of light chains, although these are usually found together with motifs in the heavy chains.[3,23] Interestingly, AGS do not accumulate in the non-functional IGHV of FL cells, confirming that they are positively selected for the functional allele.[3] AGS are found in both IgM+ cases (90%) and IgG+ cases (73.5%),[22] with the lower incidence in the latter pointing to possible heterogeneity in this subset. Location of the AGS in the IGV sequences was known to be the in the CDRs, with influences on location arising from appropriate germline sequences and mutable hotspots, but was not derived from preferential IGHV usage.[3]

Crystallographic analysis of lymphoma-derived Fabs has now revealed the importance of this positioning.[13] It turns out that the CDR sequences selected in the FL cells act as a shield surrounding the added mannoses, apparently blocking access of the relatively large α-mannosidase 1 enzyme required for the further processing of the glycan during Golgi transit (Fig.3). An illustrative case of FL selected for Fab crystallization had two heavy chain N-glycosylation sites, one in CDR1 at position N38, and one germline (GL) site in CDR2 at N57 of the IGH4-34 V region, which appears to never undergo N-glycosylation[23] (Fig.3a). The crystal structure confirms the liquid chromatography-mass spectrometry analysis which detected oligomannoses in CDR1 and no glycan addition at the GL site.[13] Further

analysis reveals that the oligomannosylated site at N38 is buried among the CDR loops, consistent with the inability of the α-mannosidase enzymes to trim it for further processing (Fig.3b).

Even if the loops appear to lie closest to the N-acetyl glucosamine sugars, this is sufficient to affect steric access of the α-mannosidases to the proximal oligomannoses.[24] This block on processing creates a "mannose paradox" whereby oligomannoses are hidden from the α-mannosidase enzymes but remain visible to lectins. [25] It seems that FL cells are sharing a strategy with some pathogens to retain oligomannoses to capture DC-SIGN for their different purposes. For FL, the shielding by the CDRs from further modification can be considered as another step in the multifactorial balance struck during lymphomagenesis.

The steric influence is clearly confirmed by the presence of fully processed oligosaccharides in the constant region of the same Ig molecule[4,5] demonstrating that the biosynthetic route for Igs of the FL BCR passes through the normal Golgi stacks. The focusing of oligomannose insertion into "suitable" acceptor IGV positions is made even clearer from the fact that sites in the framework regions of some cases of FL (usually additional to CDR sites), or in certain subsets of DLBCL, commonly do not have oligomannose termination.[13] There may be additional advantages of the insertion of oligomannoses into the BCR of FL cells as their presence actually blocks access of the original antigen.[26] Hypothetically, such a block would prevent stimulation by antigen which would drive unwanted differentiation.

The CDR location is therefore required for consequential restriction to oligomannosylation of the BCR in FL, and can be distinguished from the addition of glycan to the framework IGV regions of certain autoantibodies, such as the anti-citrullinated protein antibodies found in rheumatoid arthritis.[27] These commonly acquire AGS in the IGV sequences, which are largely located in the framework regions and are fully glycosylated with addition of terminal sialic acids.[27] This

underlines the importance of positioning of the AGS which is strikingly different from FL. The function of the glycans in autoimmune disease remains speculative but, in chronic autoimmune conditions, an influence on selection of certain autoantibodies has been proposed.[28]

The essential nature of the oligomannose insertion for FL is supported by the finding that, despite ongoing somatic hypermutation, AGS are retained by all individual emerging clones during the course of the disease.[29] In the rare case where a particular motif may be lost, another emerges in a different position. AGS are also present in FL cells located in separate tissue sites and following transformation,[29] raising the question of the incidence and positioning of AGS in DLBCL.

Diffuse Large B-Cell Lymphoma (DLBCL)

From an earlier analysis, AGS were detectable in 13/32 cases of DLBCL,[3] but the more recent subdivision of this lymphoma based on transcriptomic analysis[30] has now allowed correlation of AGS incidence among large numbers of cases of newly defined subtypes.[13] As might be anticipated from the fact that GCB-DLBCL is known to be closer to FL,[30] motifs were highly associated with this category (60%+), and were rarer in ABC-DLBCL (13%+) or in the "unclassified" subtype (24%+). Critically, the vast majority (87%) of motifs in GCB-DLBCL were located in the CDRs, as in FL, whereas >50% of those few found in ABC-DLBCL were in framework regions.[13] Interestingly, in the minor subset of FL which is t(14;18)-negative, AGS were also found only in framework regions.[31] This location, and the tendency to over-use the IGHV4-34 gene, connects these cases to ABC-DLBCL.[32,33] With regard to IGHV4-34, as mentioned already, there is a germline AGS sequence in the IGHV CDR2 but since this is not N-glycosylated it can be discounted as a glycan-mediated influence.[23]

Among the genetically defined subgroups of GCB-DLBCL, 74% of the AGS+ cases with CDR location were classified as EZB.[13] This is expected given that EZB cases are defined by a BCL2 translocation and by mutations in epigenetic regulators, features characteristic of FL. Adding in the presence of AGS draws all the features of FL together, consistent with an origin of a proportion, but not all, of EZB cases from FL. Equally some cases with AGS did not align with EZB. Although there is strong overlap with the EZB category, the presence of AGS in CDRs could allow more definition of the FL-derived subtype. This could be of clinical value since preliminary assessment of DLBCL revealed that the cases within the EZB subtype which mirrored FL in having AGS in the CDRs, progressed more rapidly than those without these AGS.[13] This distinction held even when cases with MYC translocation were removed, although numbers then were small and further studies are required.

Recently, a transcriptionally defined microenvironmental influence has been revealed as potentially acting on the genetic subdivisions of DLBCL, with possible clinical relevance.[34] It will be very interesting to map the AGS patterns on to the new categories and this is in progress.

Burkitt's Lymphoma

Burkitt's lymphoma (BL) is generally considered to originate from the dark zone of the germinal center. It comprises two main categories; endemic BL, mainly found in sub-Saharan Africa, and highly associated with Epstein Barr Virus (EBV); and sporadic BL, less commonly associated with EBV and including cases associated with immunodeficiency such as HIV/AIDS.[35] Both categories carry chromosomal translocations involving the MYC oncogene on chromosome 8 and the Ig locus, the most common being t(8;14).[35] However, as for FL, in spite of the loss of one allele, the majority of cases retain expression of surface Ig, suggesting involvement of the BCR in a pathogenesis very different from FL. In a few cases of sporadic BL, a drive

by modified autoantigens has been reported,[36] but in others, there is evidence for an antigen-independent "tonic" stimulation.[37]

A survey of the IGHV genes for N-glycosylation (AGS) sites revealed a high incidence in endemic BL (14/17 cases) and a significant but lesser incidence in sporadic BL (10/23 cases) which tends to have lower levels of somatic hypermutation.[38] In terms of location of sites, ~50% of each BL category were in the CDRs, pointing to a similarity to FL, possibly in lectin dependence. The presence of AGS adds discrimination between tumor types, illustrated by the heterogeneity within DLBCL; and by the very low incidence of AGS in MALT lymphoma, likely derived from marginal zone B cells[38] It will be of interest to probe for oligomannosylation in the BCR of BL cases to assess potential influences of a microenvironmental lectin on these aggressive tumors.

DC-SIGN

The major function of DC-SIGN is to recognize pathogens. It is part of the "spy network" of protection against infection[39] and is expressed by subepithelial dendritic cells, by follicular dendritic cells (Fig.2) and by M2-polarized macrophages. DC-SIGN binds to oligomannoses expressed by a range of infectious organisms including viruses such as HIV-1, mycobacteria and fungi. In HIV-1 infection, DC-SIGN-expressing dendritic cells ferry virus, bound via the highly mannosylated gp120, from the mucosa to T cells for delivery via CD4/CCR5. In this regard it is acting as a trans receptor, which does not activate the ferrying cells directly although it can modulate other activation pathways, potentially leading to immunosuppression.[40,41]. Evidently this relatively quiet anchoring and supportive function of DC-SIGN-expressing cells is captured by lymphoma cells for their own ends.

Structurally DC-SIGN is a Type 2 (C-terminus is exposed outside the lipid bilayer) membrane protein which forms stable tetramers. It is a lectin, which binds target sugars via four calcium-dependent carbohydrate recognition domains (CRDs)[39] linked to oligomerized neck regions (Fig. 1b). The clustered domains generate a multivalent glycan-binding surface with each CRD possessing a Glu-Pro-Asn motif that preferentially binds to oligomannose-type saccharides.[39]

In common with other lectins, DC-SIGN has a relatively low affinity for the target oligomannoses, relying on multivalency for increasing avidity. Understanding the operation of sIg-Mann interactions with DC-SIGN requires new thinking since, in contrast to most protein receptor-ligand interactions, binding does not function as a deterministic on/off switch but rather as a progressive tuning of a cell-cell interaction (Fig.4a).[39]

Interaction of DC-SIGN with lymphoma cells

It is difficult to mimic *in vitro* the low affinity/high avidity interactions likely to occur between cells *in vivo*. However, the tetrameric DC-SIGN-Fc soluble reagent binds specifically to FL-expressed Ig-Mann[5] and induces activation of upstream BCR signaling responses,[5,12,42] comparable to that induced by anti-IgM, but at a lower level. Despite the weaker response, DC-SIGN is able to induce a similar downstream transcriptional response to anti-IgM in cell lines and in primary FL cells.[42] The pathways activated included those associated with B-cell survival, proliferation and cell-cell communication. In addition, engagement of DC-SIGN mediates intracellular cross-talk via the BCR, downregulating expression of chemokine receptors such as CXCR4.[42]

FL cell-expressed sIg-Mann can also directly interact with DC-SIGN+ cells, either via a transduced cell line or via cells matured from monocytes by GM-CSF/IL-4, forming clusters which could be broken by a MoAb directed at the carbohydrate-

recognition domains[43,13] (Fig.4a). More recently we have found that engagement of the BCR by DC-SIGN induces adhesion of FL cells to the integrin ligand VCAM-1, via proximal kinases and actin regulators.[15] Since FDCs express VCAM-1[44] which is retained in FL,[16] this could promote beneficial adhesion of FL cells to FDCs.

Despite some similarities, there is a critical difference between the effects of anti-IgM and DC-SIGN on FL cells. Binding of high affinity anti-IgM gives a strong signal to B cells and leads to endocytosis and death, the latter only partly ameliorated by BCL-xL.[45] A similar BCR-induced death occurs in B cells from chronic lymphocytic leukemia, and is not prevented by BCL-2.[46] A parallel phenomenon is seen in a mouse model where normal memory B cells are silenced by engaging high-affinity antigen in the absence of T cells.[47] These findings illustrate that B cells are vulnerable to BCR-induced death unless protected by T cells, possibly reflecting a mechanism to control antibody production.

In contrast, DC-SIGN binding does activate BCR pathways in FL cells but does not induce endocytosis or death,[13,15] thereby providing an ideal protected environment with a "Goldilocks" intermediate signaling level for tumor cells to survive and proliferate(Fig.4b). The Goldilocks principle is common in biology and refers to a balancing act, where structural stability and functional properties are poised at values that are just right for the environment the cell faces. Tumor cells are no exception.

However, although binding of DC-SIGN to sIg-Mann on FL cells does not induce endocytosis, it does modulate BCR function. Pre-treatment of either FL-derived cell lines or primary FL cells with DC-SIGN appears to paralyze the BCR, so that it is unable to respond to stimulation via anti-IgM.[42] This is not due to blockade but seems instead to be caused by a nanoscale reorganization of surface IgM-Mann rendering it less able to signal.[48] The effect is rapidly reversible in vitro so unlikely to be mediated by transcriptional changes. Lectin-mediated modulation of

receptor function is reminiscent of the effects of galectin on T-cell function, and has been explained there as due to formation of a galectin lattice which "freezes" receptor function.[49] The effect of DC-SIGN may be yet another strategy for protecting FL cells from BCR-mediated stimulation by antigen.

Concluding remarks

The mandatory insertion of mannoses into the antigen-binding site of FL cells points to the importance of this post-translational feature for lymphomagenesis. The sequence of events which incorporates this change in the nature and function of the BCR is shown in Fig.5. Insertion of oligomannose occurs early and appears to be a critical step towards full-blown FL. Together with upregulation of BCL2 it allows the cell to persist and to accumulate other mutations, often in epigenetic regulators, which then drive the cell to further escape from control.[50] Some of these mutations in chromatin-modifying genes, such as CREBBP, occur before overt disease.[51] A multi-step pathway is likely to occur in all relatively indolent tumors, but it is well illustrated in FL. The BCR modification occurs in all tissues analyzed although the motif can differ, in line with independent events in separate sites. However it is not just a preliminary event as it persists over time, even being carried through transformation. Since every case of FL has a different IGV sequence, the AGS will vary in number and position, raising the question whether this variation grades the ability of the FL cell to interact with the microenvironment. A correlation between the ability to bind to DC-SIGN *in vitro* and clinical behavior would be a simple starting point to answer this question.[12] There could also be an association with transformation to a subset of DLBCL, thereby adding to the predictive power of the genetic background.[52]

The function of this structural change in the BCR is beginning to emerge: the inserted oligomannoses have multiple potential roles including protective

persistent interaction with DC-SIGN, blockade of antigen access and downmodulation of BCR function. While these functions have been documented *in vitro,* it now turns out that they match observations *in vivo*. DC-SIGN is plentiful in FL tissues, being expressed by M2-polarized macrophages largely in the interfollicular zone, but also by follicular dendritic cells, first on the FDC network and then on scattered fragments as disease progresses. The tissue localization will be presented in another review of this series by Dr Andrea Radtke, and, via a collaboration with Dr Richard Burack and Dr Yi Zhang, we have similar unpublished findings, with a preliminary example shown in Fig.2. An urgent question is whether the presence and number of either of these cell types correlates with disease behavior. The role of macrophages has been much debated in FL, with initial findings that increased numbers correlated with poorer prognosis, but a later reversal of this correlation probably due to the need for macrophages to mediate the effect of anti-CD20.[53,54] In terms of DC-SIGN, it is not possible yet to know whether FDCs or macrophages are the most important supporting cells for FL, and it could be both.

A speculative summary of how the documented effects of DC-SIGN could enable lymphomagenesis is shown in Fig.5. It is clear that B cells which carry the t(14;18) translocation are perfectly able to undergo somatic hypermutation, and presumably antigen engagement. For normal B cells and likely for the cells expressing BCL-2, the introduction of sites for N-glycosylation will occur during SHM, but these are not generally retained. The reason could be that oligosaccharides could block antigen access, thereby opposing antigen selection, and leading to death of unselected cells. However, given the generation of sites in the CDRs which are “just right” for termination at oligomannoses, these cells free themselves of reliance on antigen by capturing DC-SIGN-expressing FDCs and benefitting from their anti-apoptotic support. It could therefore be seen as a rescue of B cells unable to bind antigen and therefore officially to be rejected. Further help from M2-macrophages would be on offer and could operate on cells

in transit through the tissue. The mechanism of support appears to include a nourishing low-level BCR signal coupled to protection against strong stimulation which in normal B cells would drive differentiation/death. Downregulation of MHC Class II in FL would similarly protect against provision of T-cell help, again holding the cells back from unwanted differentiation.[55] The scene would be set for accumulation of mutations which drive lymphoma development,[56]

While the focus here is on the critical interactions between the FL BCR and DC-SIGN-expressing cells, there are many other influences operating in the FL tissue, which are discussed in detail in an accompanying Review of this Series from Karin Tarte's laboratory (Laurent C et al). Unravelling the biology should reveal new potential targets to add to current therapy and there is a clinical aspect of the DC-SIGN interaction which is worth exploring. Antibodies against the CRD of DC-SIGN are available already, having been made for other purposes,[57] and more are being produced. The aim would be to simply block the sIg-Mann-DC-SIGN interaction seemingly required by FL cells, ideally at an early stage of disease, although retention of sites suggests that reliance on DC-SIGN continues through disease. DLBCL is another story, and while there is a clear subset which carries oligomannosylated CDR-located sites, it remains to be seen if this is just a useful marker, or if antibody would be effective there also. For FL which is surrounded by an overwhelmingly heterogeneous population of T cells and stromal cells, as will be described in the other reviews in this Series, the tumor cells are telling us that they cannot proceed without oligomannosylated BCRs.

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Conflict of interest disclosure

FKS and FF declare no competing conflicts of interest.

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Figure legends

Figure 1: Natural and introduced N-glycosylation sites in human IgM

a) Domain structure of normal human IgM with natural germline sites for N-glycosylation (N-X-S/T) indicated as red hexagons at amino acid positions in the constant region. Introduced sites in the variable region of cases of follicular lymphoma are shown as green diamonds

Available oligomannoses are specifically found in the variable region as branched mannoses linked to N-acetyl glucosamine

b) DC-SIGN (dendritic cell-specific intercellular adhesion molecule 3 grabbing non-integrin) is a tetramer stabilised by the neck region. It interacts with oligomannoses introduced into the antigen-binding site of FL cells, via its carbohydrate-recognition domains.

Figure 2: Expression of DC-SIGN in follicular lymphoma (FL) tissue.

A representative case of FL is shown without (left panel) or with (right panel) staining with anti-DC-SIGN by immunofluorescence. In the left panel, M2 macrophages, mainly in the inter-follicular area, are stained red with anti-CD163, and follicular dendritic cells (FDCs) in the neoplastic follicles outlined in dotted white lines are stained blue with anti-CD23. In the right panel, M2 macrophages co-stained with green anti-DC-SIGN appear as yellow. FDCs co-stained with green anti-DC-SIGN appear as cyan.

Figure 3: An illustrative example of a case of FL to reveal the nature and structure of the glycan-modified Ig variable (IGV) region.

a) Site-specific glycan composition was determined by liquid chromatography-mass spectrometry and oligomannoses at N38 in complementarity-determining region-1 of the Ig heavy chain (HCDR1) are depicted in green. There were no sites in the Ig light chain. The gray circle at the germline position N57 indicates no detectable glycan addition.

b) Crystals of the derived Fab' were analyzed at 1.65Å resolution and the CDRs of the Ig heavy chain (HCDR) are colored as indicated. The glycan is shown as two resolved N-acetyl glucosamine residues at position N38 which are attached to the oligomannoses. More detail is available in ref 13.[13]

Figure 4: Consequences of interaction of DC-SIGN with the mannosylated B- cell receptor (BCR) of FL cells.

a) The clustering of DC-SIGN-expressing cells with oligomannoses in the BCR of FL cells can be blocked by an anti-DC-SIGN antibody against the carbohydrate-recognition domain.[13]

b) Interaction of FL cells expressing sIg-Mann with DC-SIGN *in vitro* generates a low level BCR signal, membrane remodeling, increased adhesion and blockade of antigen access, [12,42,15,26] but no detectable endocytosis or apoptosis.[13,15]

Figure 5: The potential influence of the mandatory oligomannoses inserted into the BCR during development of FL.

The first event in the bone marrow is the t(14;18) translocation which upregulates BCL2. These B cells than then apparently undergo somatic hypermutation (SHM) and antigen selection. There is a likely bifurcation of cells able to bind antigen and those which have acquired oligomannoses in the BCR which cannot undergo antigen selection. These "rejects" may be lost or could be rescued by DC-SIGN on FDCs and/or macrophages, forming a population awaiting mutations in chromatin-modifying genes (CMG) to drive lymphomagenesis.

Insertion of oligomannose into the B-cell receptor of follicular lymphoma cells enables DC-SIGN-mediated microenvironmental support

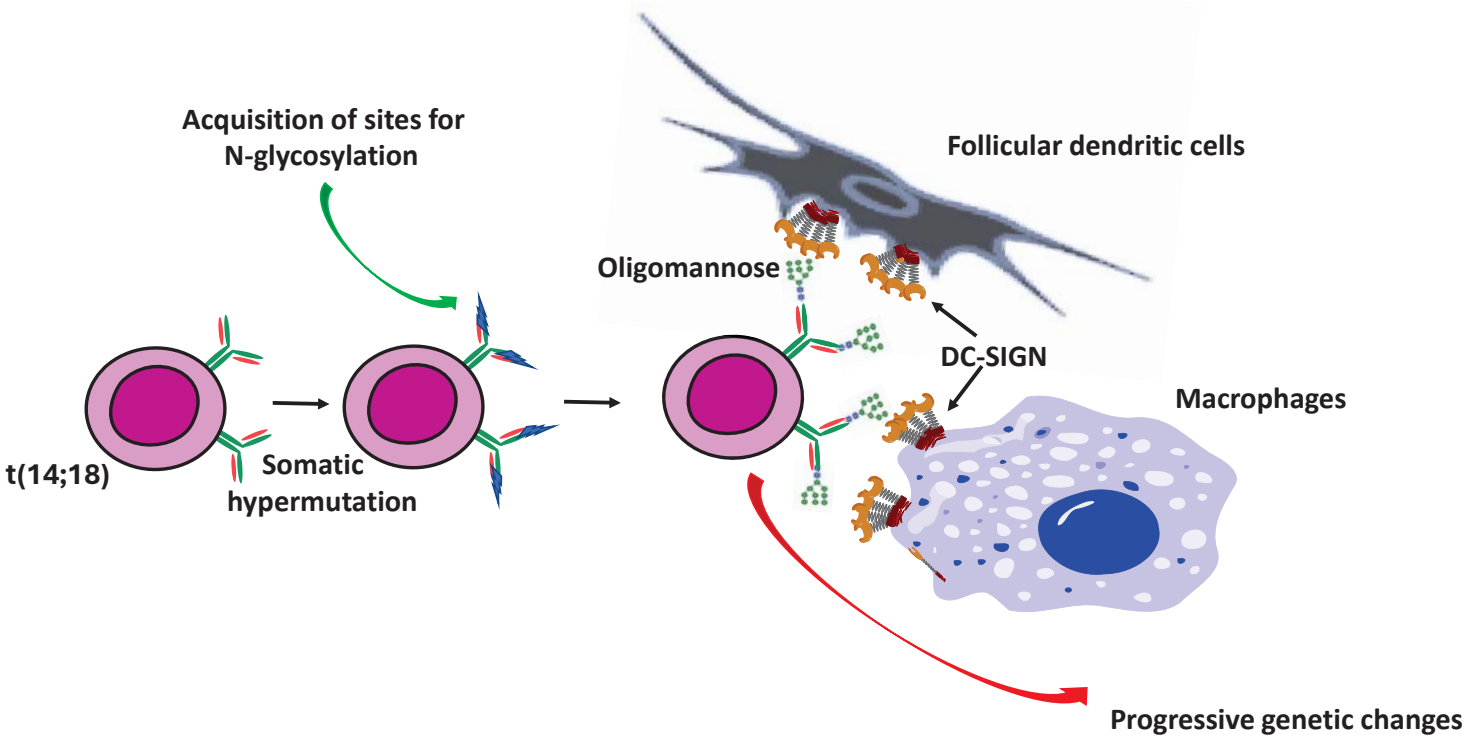


Fig.1a

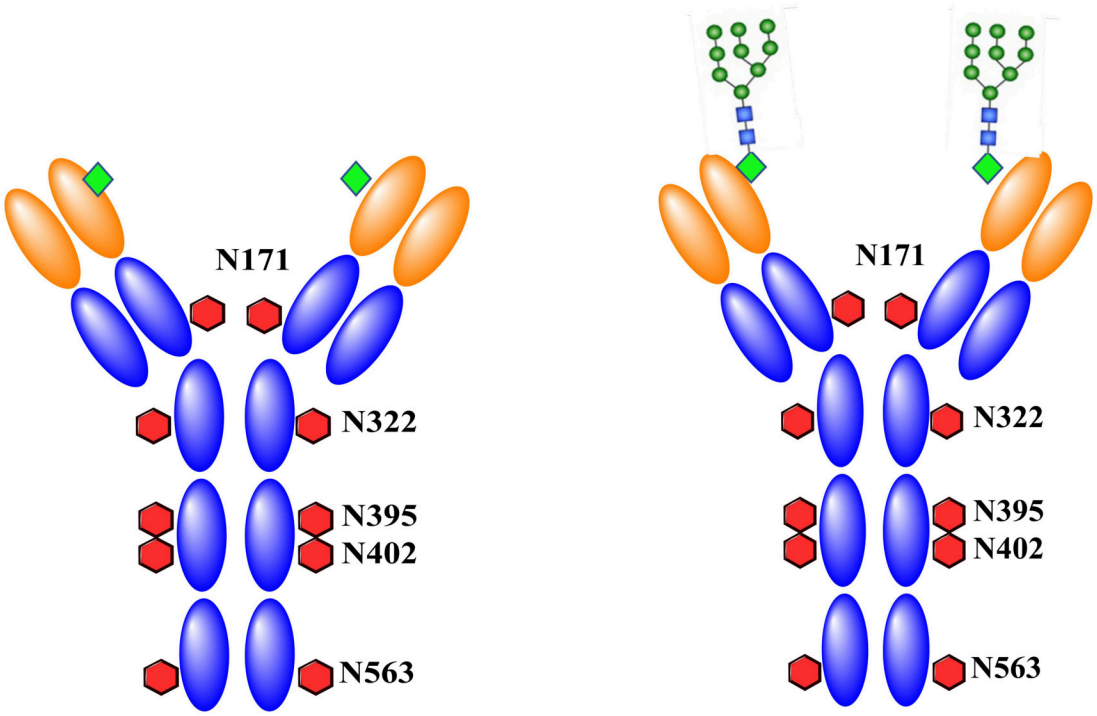

N171
N322
N395
N402
N563
N171
N322
N395
N402
N563

Fig.1b

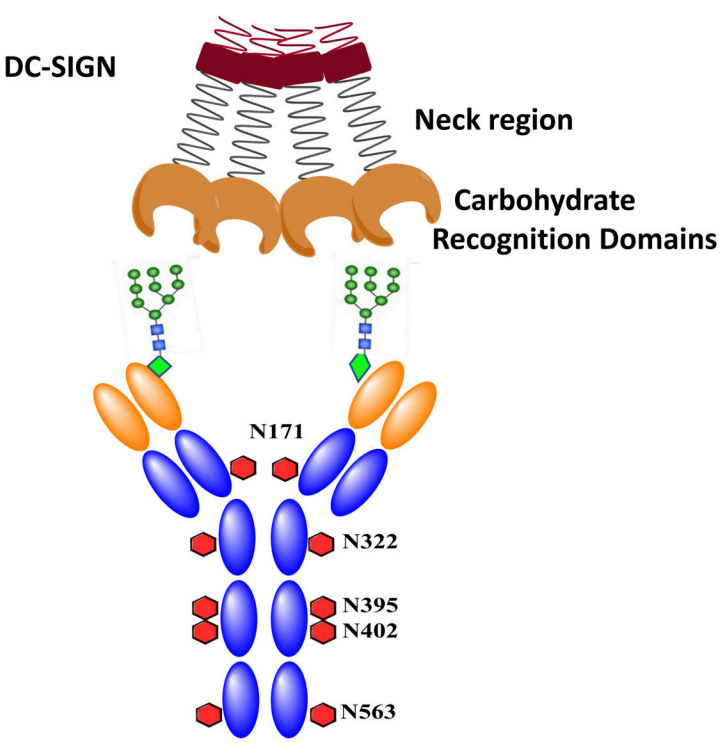

DC-SIGN
Neck region
Carbohydrate
Recognition Domains
N171
N322
N395
N402
N563

Fig.2

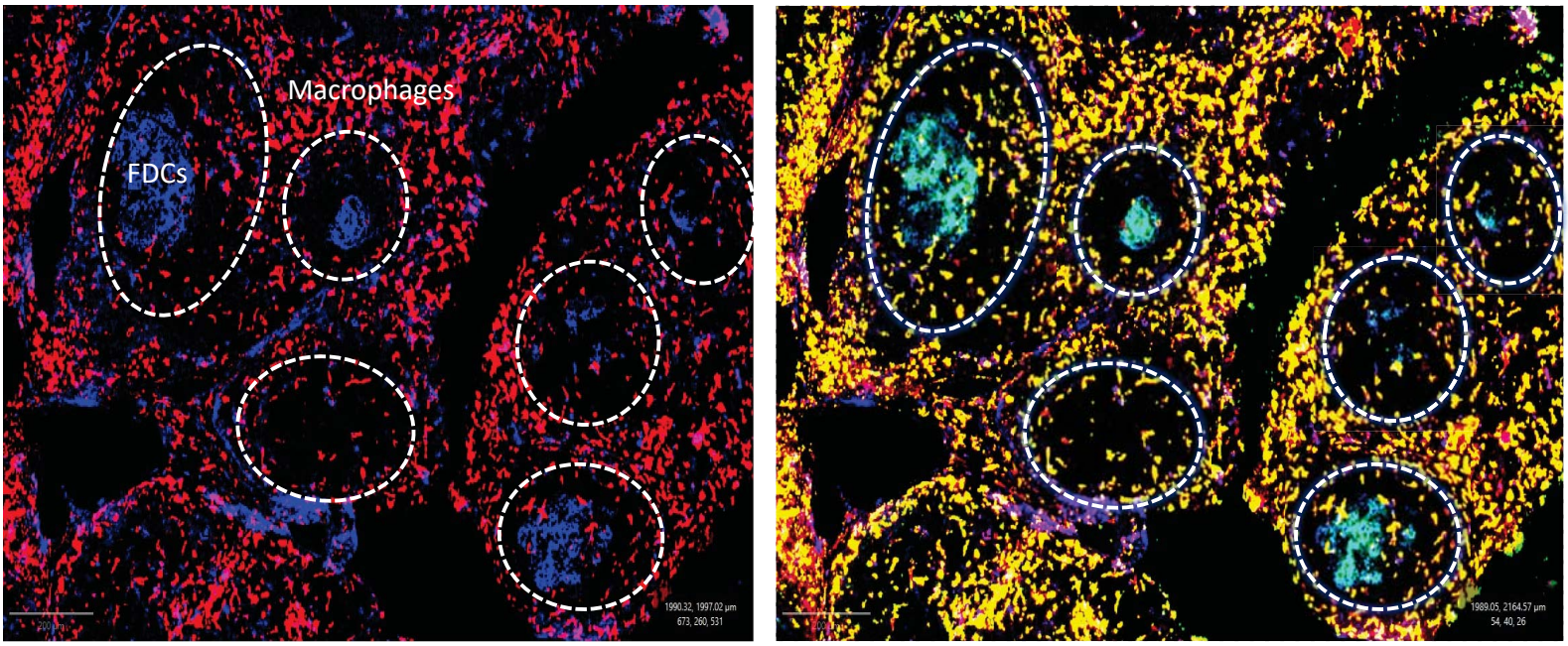
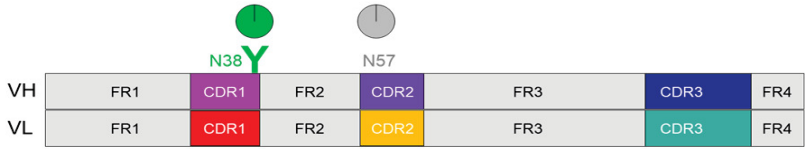

Macrophages
FDCs
1990.32, 1997.02 µm
673, 260, 531
1989.05, 2164.57 µm
54, 40, 26

Fig.3

a)


N38
N57
FL14
VH
VL
FR1
CDR1
FR2
CDR2
FR3
CDR3
FR4
FR1
CDR1
FR2
CDR2
FR3
CDR3
FR4

b)

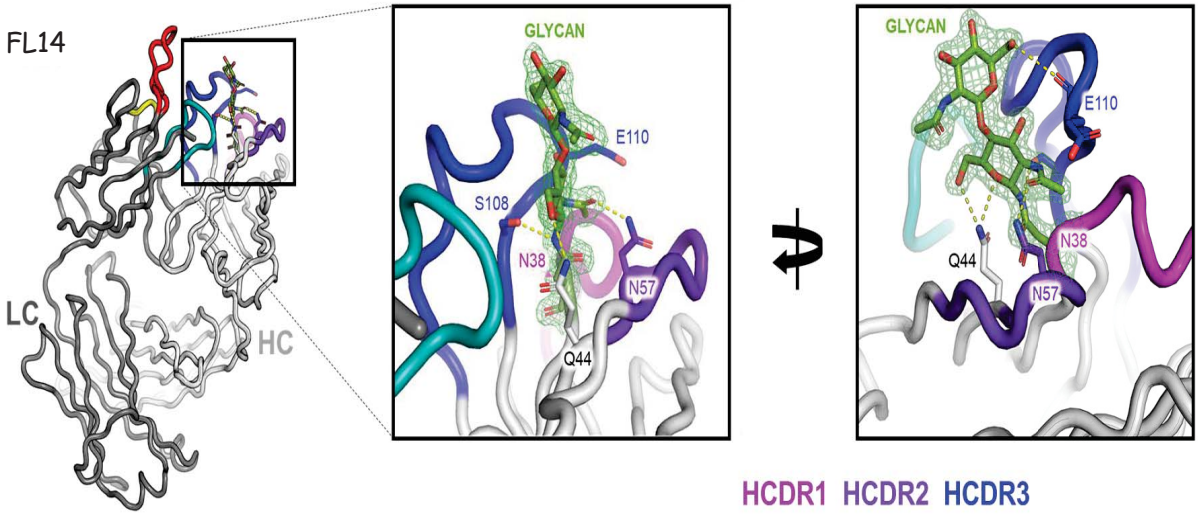

FL14
LC
HC
GLYCAN
E110
S108
N38
N57
Q44
GLYCAN
E110
N38
Q44
N57
HCDR1 HCDR2 HCDR3

Fig.4a

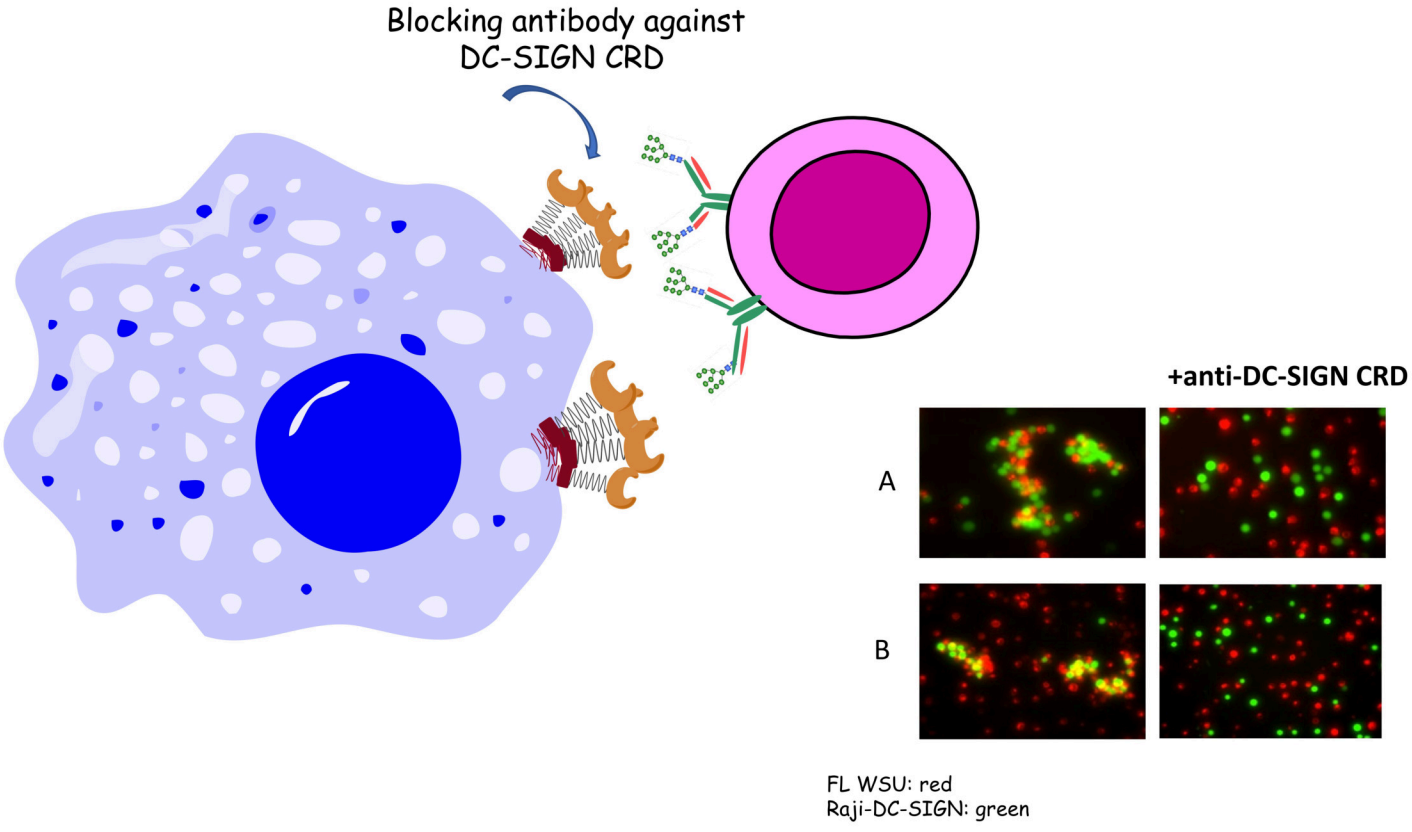

Blocking antibody against
DC-SIGN CRD
+anti-DC-SIGN CRD
A
B
FL WSU: red
Raji-DC-SIGN: green

Fig.4b

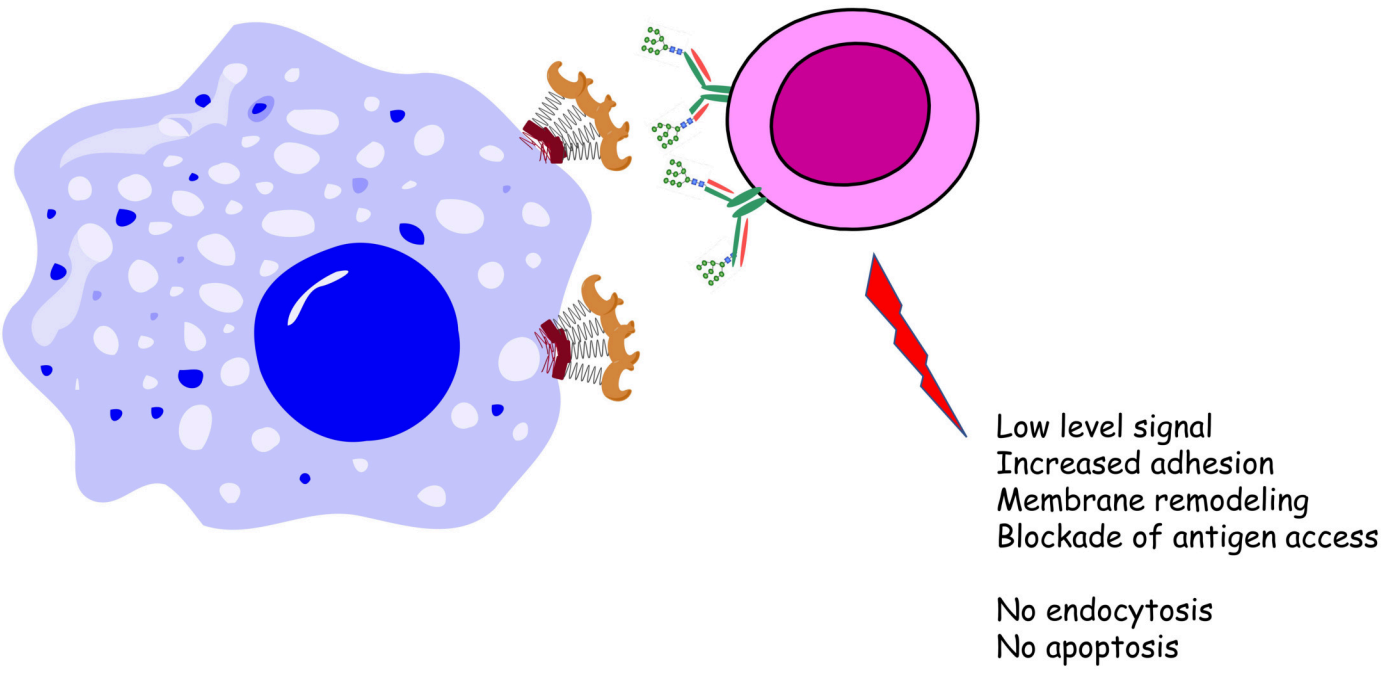
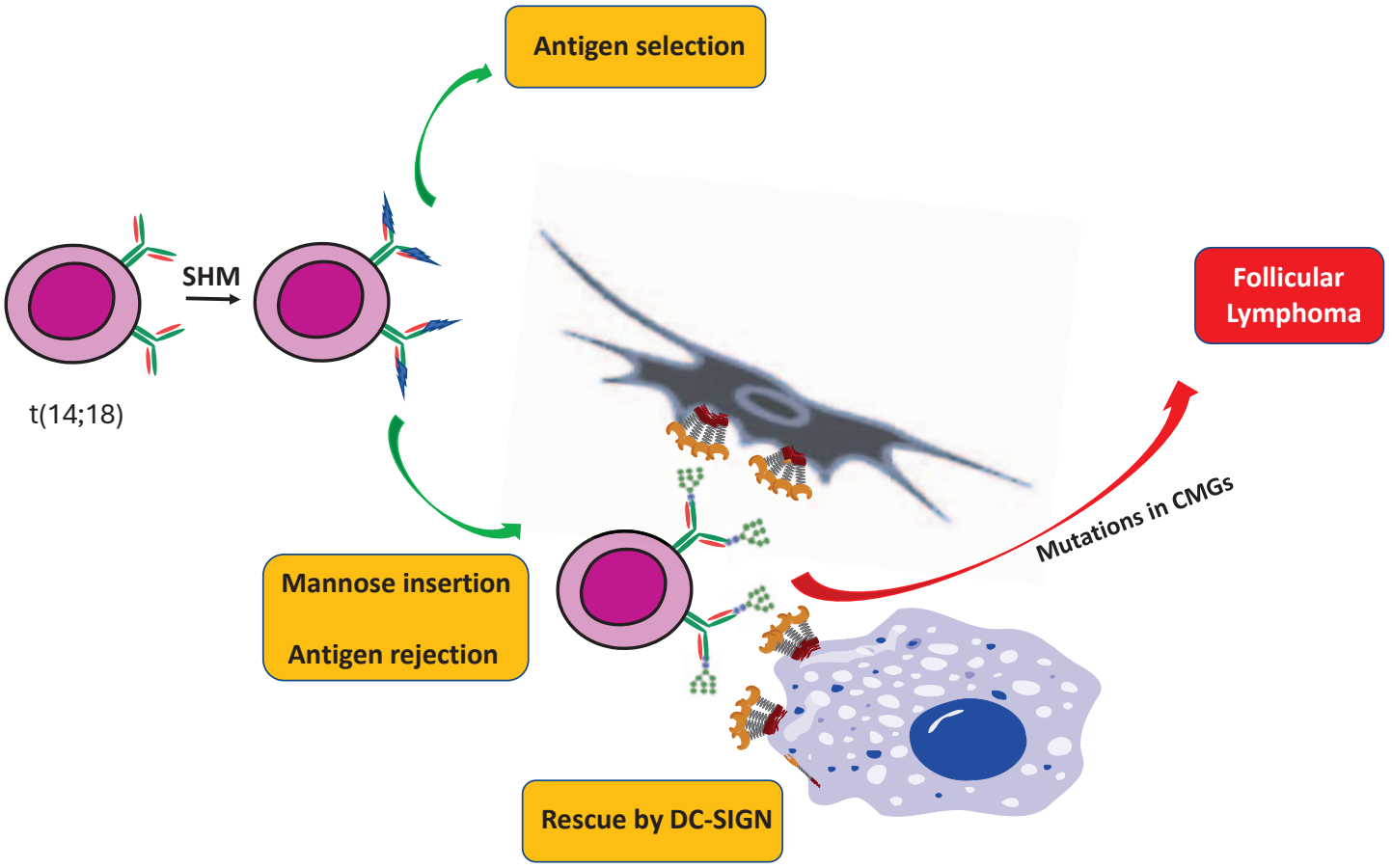

Low level signal
Increased adhesion
Membrane remodeling
Blockade of antigen access
No endocytosis
No apoptosis

Fig. 5


Antigen selection
SHM
t(14;18)
Follicular Lymphoma
Mutations in CMGs
Mannose insertion
Antigen rejection
Rescue by DC-SIGN