Supplementary material

Methods

Biopsies and arthroscopic knee evaluation

Synovial tissue biopsies (of <1mm in diameter) were obtained during knee arthroscopy using 3.5mm grasping biopsy forceps under direct vision using a Hopkins 2.7mm 300 arthroscope, then snap frozen in OCT compound (cohort-1) or fixed and paraffin embedded (cohort-2). Arthroscopic visual analogue scores (arVAS) were used as a measure of macroscopic joint inflammation (scale between 0 and 100) (1).

Tissue culture of synoviocytes and cytokine stimulation of IL-7 expression

Synovial tissue was digested and primary cultures established (2). At passage 3, cells were treated for 16 hours with cytokines: IL-1-beta, IL-6, IL-10, TNF-alpha (all 25 ng/ml), IFN-gamma (10ng/ml), TGF-beta1 (20ng/ml) (all R&D, Abingdon, UK).

IL-7 expression was measure by qPCR. RNA was extracted as previously described (3) and first-strand cDNA synthesised using High-Capacity cDNA Reverse Transcription Kit. Real-time qPCR was performed using an ABI Prism 7900 sequence detection system (Applied Biosystems, Warrington, UK) in the presence of SYBR-green. Transcription of IL-7 was normalised to *GAPDH*. Primers were designed using Primer Express-v.2 (Applied Biosystems). Primer concentrations used were 500 nM for all (Table S1).

Gene expression

For cohort-1, shavings including frozen solid biopsy tissues (~1mm in diameter) were recovered to eliminate as much OCT as possible, washed in PBS and then placed in guanidine-based lysis buffer and manually homogenised. Lysates were then filtered (0.7µm) and RNA extracted using a standard phenol:chloroform method.

Shavings from cohort-2 (formalin fixed/wax embedded tissues) were collected and RNA was recovered using the RNeasy FFPE Kit from QIAGEN according to manufacturer's instruction. DNase treatment (DNA-free) was performed prior to reverse transcription with High-Capacity cDNA Reverse Transcription Kit (Life Technologies) and application of cDNA (together with Gene Expression Master Mix, Life Technologies) to the TaqMan arrays. The arrays were run on a 7900HT Sequence Detection System according to the manufacturer's recommendations (Life Technologies).

Bisulphite sequencing

Genomic DNA (500ng) was bisulphite converted using the Zymo Research EZ DNA Methylation Kit (Cambridge Bioscience, Cambridge, UK) following the manufacturer's instructions. PCR amplification of 10 fragments of the IL-7 gene comprised 5µl of Qiagen HotStarTaq Master Mix (Qiagen, Crawley, UK), magnesium chloride (2mM), forward and reverse primers (0.2µM each, sequences in Table S1), DNA in a final volume of 10µl. Thermal cycling conditions were 94°C for 12 minutes followed by 40 cycles of 94°C for 10 seconds, 55°C for 20 seconds and 72°C for 30 seconds. PCR reactions were prepared for sequencing using the BigDye Terminator v.1.1 (Life Technologies) using 2.5µl of PCR product. Sequencing reactions were cleaned by ethanol precipitation, resuspended in 20µl of HiDi formamide (Life Technologies) and run on an Applied BioSystems 3130xl Genetic Analyser. Sequencing data was analysed by visual inspection on Applied BioSystems Sequencing Analysis Software v.5.2.

H&E staining

Tissue for light microscopy was fixed in 10% buffered formalin. Sections were de-waxed in xylene and ethanol, then washed in water and stained with Mayer's haematoxylin and counterstained in 1% aqueous Eosin Y, after which they were dehydrated in ethanol, cleared in xylene and mounted in DPX, all according to routine pathology procedures.

Immunohistochemistry (IHC)

Paraffin embedded sections were de-waxed with Access Super solution and staining was performed using X-Cell plus kit (Menarini Diagnostics Ltd., Berkshire, UK). Slides were incubated in peroxidase block buffer followed by casein block solution for 10 minutes. Incubation with monoclonal antibody CD3 (rabbit monoclonal, clone SP7, Abcam, dilution 1:200), CD68 (rat monoclonal, clone ab53444, Abcam, dilution 1:100), CD20 (goat polyclonal, clone M-20, Santa Cruz, dilution 1:200) or IL-7 (mouse monoclonal, clone MAB207, R&D Systems, dilution 1:200) was performed for 1 hour followed by three washes, then application of the Universal probe reagent for 30 minutes. Slides were incubated in X-Cell Polymer HRP reagent for 30 minutes prior to adding 3, 3'-diaminobenzidine (DAB) solution for 5 minutes and counterstaining in haematoxylin for 2 minutes. Finally, slides were dehydrated through ethanol and mounted in di-N-butyl phthalate xylene.

Automated lymphocyte infiltration scoring

Slides were examined under bright-field mode using a multispectral Nuance camera (PerkinElmer, London,

Table S1. Primers sequences.

Gene expression		
IL-7	GGTATATCTTTGGACTTCCTCCCCT	AACACTCTCATATTGTTTGCCATCTT
GAPDH	AACAGCGACACCCACTCCT	CATACCAGGAAATGAGCTTGACAA
bisulphite sequencing		
Set	**Left primer (5'-3')**	**Right primer (5'-3')**
1	GGAGGTTGAGGTGGGTAGATTAT	AAAACAATCATATAAAAAAATTCCTT
2	TTTAGTTTTTTTAGGGGAAGTAAGG	TAACATTATTATCACACCCACACTC
3	TGGGAAGGTAATTTATGTTTTTAATG	AAACTAAAAAAAACCAAATCAAATC
4	AGATTAGGGTTTTGGGAGTGATTAT	ACCCATAAAAAAACCAATACTCTCC
5	AAGTAGTTAAAGGTTTTTTGTTTGG	ACCTATTCCCCTAAAATTTAAAAAC
6	AGATTTTATGTTGATGATTTTAAAG	AAATAATCTCTACAACTAATTCCTCT
7	TTTGAGTAGGTGTATGTATAGTAGA	AATAAAAAACTTCCCAAATAATCTCTC
8	TGAGAGATTATTTGGGAAGTTTTTTAT	CCCTCTCTCCTAAACACCTACTTC
9	TGGGAAGGTAATTTATGTTTTTAATG	AAACTAAAAAAAACCAAATCAAATC
10	TTTGAGTAGGTGTATGTATAGTAGA	AATAAAAAACTTCCCAAATAATCTCTC

UK) connected to a Nikon E1000 microscope and operated using Nuance v.3.0.1.2 software (Caliper, PerkinElmer). The software enabled a series of images to be captured using 20x magnification and multispectral light between 420-720 nm. Images were automatically merged into a single image covering the whole tissue. The inForm software (Perkin Elmer) was then trained to recognise three classes of tissue structure: cells, vessels and fatty areas by exemplifying representative regions. The process was repeated until an accuracy of >95% was obtained. Areas of images that were hard to analyse due to folds in the tissue or air bubbles were manually edited.

For quantifying of CD3, CD20 or CD68 the images were analysed automatically, segregating the DAB (brown) and haematoxylin (blue) signals and measuring each area of positivity (Fig. S1B). A cell index (ratio of pixel counts for haematoxylin over total area) was calculated. The percentage of lineage positive cells was obtained by ratio of the DAB-positive area to the cell index. The lining and stromal (sub-lining) areas of the synovial tissues were scored separately using the same methodology.

Quantification of IL-7 expression using multispectral image analysis

The percentage of cells positively stained for IL-7 was determined as for lymphocyte infiltration.

The level of IL-7 was further analysed using windows of DAB intensity (bins). Unstained cells were assigned to a negative bin. Areas of low DAB intensity with no associated nuclei were considered background and were assigned to a second bin, added to the negative. DAB staining considered positive was then separated according to intra-nuclear (DAB and haematoxylin) and extra-nuclear (DAB only, accounting for cytoplasmic IL-7 and diffusion around cells) and added together. Surface of positive staining was then evaluated as a percentage of the total surface (excluding blood vessel, fatty area, air bubbles and folds in the tissue). Two scores were reported: the percentage of positive cells and total IL-7 expression.

Tables S2. Card table 48 genes.

Common name used in this paper / alternative name(s)	Gene description	Assay ID
BAX	BCL2-associated X protein	Hs99999001_m1
BCL2	B-cell CLL/lymphoma 2	Hs99999018_m1
Caspase 1 / CASP1	caspase 1, apoptosis-related cysteine peptidase	Hs00354832_m1
CD19	B-Lymphocyte Surface Antigen B4	Hs00174333_m1
CD4	T-Cell Surface Antige4	Hs00181217_m1
CD55	decay accelerating factor for complement	Hs00892618_m1
CD68	Macrophage antigen	Hs00154355_m1
CD8	T-lymphocyte differentiation antigen	Hs00233520_m1
CXCL12 / SDF-1/	chemokine (C-X-C motif) ligand 12	Hs00171022_m1
CXCL13/BCA1/ANGIE	chemokine (C-X-C motif) ligand 13	Hs00757930_m1
EGF	epidermal growth factor	Hs01099999_m1
EIF2AK3	eukaryotic translation initiation factor 2-alpha kinase 3	Hs00178128_m1
FGF1	fibroblast growth factor 1	Hs01092738_m1
ICAM1 / CD54	intercellular adhesion molecule 1	Hs00164932_m1
IgG1 – heavy chain	immunoglobulin heavy constant gamma 1	Hs00378340_m1
IgM – Heavy chain	immunoglobulin heavy constant mu	Hs00378435_m1
IgK – constant/light	immunoglobulin kappa constant,	Hs00415042_m1
IL-10	interleukin 10	Hs99999035_m1
IL-17	interleukin 17A	Hs99999082_m1
IL-1β	interleukin 1, beta	Hs99999029_m1
IL-6	interleukin 6	Hs99999032_m1
IL6ST / gp130	interleukin 6 signal transducer (gp130)	Hs00174360_m1
IL-7	interleukin 7	Hs99999033_m1
MMP1	matrix metallopeptidase 1	Hs00233958_m1
MMP3	matrix metallopeptidase 3	Hs00968308_m1
CD20/ MS4A1	B-Lymphocyte Surface Antigen B1	Hs00544819_m1
NLRP3	NLR family, pyrin domain containing 3	Hs00366465_m1
CD31/PECAM1	platelet/endothelial cell adhesion molecule	Hs00169777_m1
PYCARD/ASC/CARD5	PYD and CARD domain containing	Hs00203118_m1
RELA / NFκB-p65 subunit	v-rel reticuloendotheliosis	Hs01042010_m1
CD138 /syndecan 1	syndecan 1	Hs00896423_m1
syndecan 4	syndecan 4	Hs01120909_m1
SFRP4	secreted frizzled-related protein 4	Hs00180066_m1
STAT1	signal transducer and activator of transcription 1	Hs01014002_m1
STAT5B	signal transducer and activator of transcription 5B	Hs00273500_m1
Synoviolin-1/SYVN1	synovial apoptosis inhibitor 1, synoviolin	Hs00381211_m1
TGFB1	transforming growth factor, beta 1	Hs00998133_m1
TNF	tumour necrosis factor	Hs99999043_m1
TACI / CD267 / TNFRSF13B	transmembrane activator and CAML interactor	Hs00963364_m1
BAFFR / TNFRSF13C	BAFF-receptor	Hs00606874_g1
BCMA / TNFRSF17	B-cell maturation antigen receptor	Hs00171292_m1
APRIL / TNFSF13	A proliferation-inducing ligand	Hs00601664_g1
BAFF / BLYS / TNFSF13B	B-cell activating factor/ B Lymphocyte Stimulator	Hs00198106_m1
TP53 / p53	tumour suppressor p53	Hs01034249_m1
VCAM1/CD106	vascular cell adhesion molecule 1	Hs00365486_m1
VEGF	vascular endothelial growth factor A	Hs00900058_m1
GAPDH	glyceraldehyde-3-phosphate dehydrogenase	Hs99999905_m1
18S	eukaryotic 18S rRNA	Hs99999901_s1

The GAPDH (glyceraldehyde-3-phosphate dehydrogenase) gene was used as reference (assay: Hs99999905_m1).

Results

Tissue characterisation: 29 biopsies

OA patients tended to be older than RA (p=0.080) and gender was biased towards female in RA (p=0.120). Inflammation (arVAS) was more pronounced in RA overall (p=0.013) although the OA samples also covered a wide range of arVAS. We confirmed previous data (on n=65 samples [4]) showing the direct relationship between the degree of immune cell infiltration and local levels of inflammation in synovial tissue (arVAS and synovitis score correlation: rho=0.972, p<0.0001).

Further confirmation of defined TA structures was made using IHC for T-cells (CD3), B-cells (CD20) and macrophages (CD68, Fig. S1). B- and T-cells were clearly scattered in tissues

classed as diffuse infiltration. B-cells formed small groups of ~10 cells in some samples but were undetected in several others. Aggregates of a few 100 cells separately staining for B- or T-cell markers were characteristic of tissue classified as 'aggregate' TA. Defined architecture with clear T- and B-cell zones defined the GCL TA group. Macrophage distribution was not particularly affected by the TA and appeared localised in lining and less frequently, in stromal layers. There was no clear TA association with RA or OA samples, although in RA samples there was tendency towards more complex TA.

Gene expression profiling for TA and B-cells: card-2 (29 RA/OA biopsies)

We designed a second array to cover additional genes related to IL-7 signalling and regulation (STAT5a, IFN-gamma, TGF-beta1), B-cell maturation (RAG-1, BLIMP, AIOLOS, XBP-1) additional Ig-subtypes, differentiation markers (CD38, CD79A, CD5, CD62L), cross talk between B- and T-cells (CD80, CD86, CTLA4, CD40L), genes involved in citrullination (PADI1 and PADI4), epigenetic mechanisms and DNA repair (HDACs, GADD34, GADD153) or bone turnover (TRAIL, OPG, RANK and RANKL).

Several genes including CD19 were detectable in less than 50% of biopsies and were removed, leaving 82 genes in the array dataset.

References

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2. JONES E, ENGLISH A, CHURCHMAN S, D. KOUROUPIS D *et al*.: Large-scale extraction and characterization of CD271+ multipotential stromal cells from trabecular bone in health and osteoarthritis: Implications for bone regeneration strategies based on uncultured or minimally cultured multipotential stromal cells. *Arthritis Rheum* 2010; 62: 1944-54.
3. JONES E, CHURCHMAN SM, ENGLISH A *et al*.: Mesenchymal stem cells in rheumatoid synovium: Enumeration and functional assessment in relation to synovial inflammation level. *Ann Rheum Dis* 2010; 69: 450-7.
4. GOËB V, WALSH C, REECE RJ, EMERY P, PONCHEL F: Potential role of arthroscopy in the management of inflammatory arthritis. *Clin Exp Rheumatol* 2012; 30: 429-35.

Table S3. Card table 96 genes.

Common name used in this paper / alternative name(s)	Description	Assay ID
BAD	BCL2-associated agonist of cell death	Hs00188930_m1
BAX	BCL2-associated X protein	Hs99999001_m1
BCL2	B-cell CLL/lymphoma 2	Hs99999018_m1
CCL21	chemokine (C-C motif) ligand 21	Hs99999110_m1
CD19	B-Lymphocyte Surface Antigen B4	Hs00174333_m1
CD38	cyclic ADP ribose hydrolase	Hs00277045_m1
CD4	T-Cell Surface Antigen T4	Hs00181217_m1
TRAP CD40LG	CD40 ligand	Hs00163934_m1
CD5	Lymphocyte Antigen T1	Hs00204397_m1
CD55	decay accelerating factor for complement	Hs00892618_m1
CD68	Macrophage Antigen	Hs00154355_m1
CD79a	immunoglobulin-associated alpha	Hs00998119_m1
CD80	Co-stimulation B7.1	Hs00175478_m1
CD86	Co-stimulation B7.2	Hs99999104_m1
CD8	T-Lymphocyte Differentiation Antigen T8	Hs00233520_m1
COMP	cartilage oligomeric matrix protein	Hs00164359_m1
CTLA-4 / CD152	cytotoxic T-lymphocyte-associated protein 4	Hs03044418_m1
CXCL12 / SDF-1	chemokine (C-X-C motif) ligand 12 (SDF-1)	Hs00171022_m1
CCL13 / BCA1 / ANGIE	chemokine (C-X-C motif) ligand 13	Hs00757930_m1
DDIT3	DNA-damage-inducible transcript 3	Hs99999172_m1
DNAJB9	DnaJ (Hsp40) homolog, subfamily B	Hs01052402_m1
DNMT1	DNA (cytosine-5-)-methyltransferase 1	Hs00945899_m1
EGF	epidermal growth factor	Hs01099999_m1
ERP44	endoplasmic reticulum protein 44	Hs00383195_m1
FGF1	fibroblast growth factor 1 (acidic)	Hs01092738_m1
HDAC1	histone deacetylase 1	Hs02621185_s1
HDAC2	histone deacetylase 2	Hs00231032_m1
HDAC3	histone deacetylase 3	Hs00187320_m1
HDAC4	histone deacetylase 4	Hs00195814_m1
HDAC7	histone deacetylase 7	Hs00248789_m1
HIF1-α	hypoxia inducible factor 1, alpha subunit	Hs00936368_m1
HSP90	heat shock protein 90kDa alpha	Hs00743767_sH
HSP72	heat shock 70kDa protein 1A	Hs00359163_s1
BIP	heat shock 70kDa protein 5	Hs99999174_m1
ICAM/CD54	intercellular adhesion molecule 1	Hs00164932_m1
IFN-γ	interferon, gamma	Hs99999041_m1
IGHD	IgD- heavy chain	Hs00378878_m1
IGHG1	IgG1 – heavy chain	Hs00378340_m1
IGHV4-	IgG4 – heavy chain	Hs00378230_g1
IGHG3	IgG3 – heavy chain	Hs00382386_m1
IGHM	IgM – heavy chain	Hs00378435_m1
IGKC	IgK – constant/light chai	Hs00415042_m1
IKZF3 / Aiolos	IKAROS family zinc finger 3 (Aiolos)	Hs00232635_m1
IL-10	interleukin 10	Hs99999035_m1
IL-17	interleukin 17A	Hs99999082_m1
IL-18	interleukin 18	Hs99999040_m1
IL-1β	interleukin 1, beta	Hs01555413_m1
IL-6	interleukin 6	Hs99999032_m1
gp 130/IL-6ST	interleukin 6 signal transducer (gp130)	Hs00174360_m1
IL-7	interleukin 7	Hs99999033_m1
ITGAX / integrin / CD11C	integrin, alpha X	Hs01015070_m1
LGALS3 / galectin 3	lectin, galactoside-binding, soluble, 3	Hs00173587_m1
LTR	lymphotoxin beta receptor	Hs00158922_m1
Ki67	antigen identified by antibody Ki-67	Hs00606991_m1
MRC1 / CD206	mannose receptor, C type 1	Hs00267207_m1
CD20 / MS4A1	B-Lymphocyte surface Antigen (transcript 1)	Hs00544819_m1
CD20 / MS4A2	B-Lymphocyte surface Antigen (transcript 2)	Hs00175091_m1
NTAN1	N-terminal asparagine amidase	Hs00386149_m1
PADI1	peptidyl arginine deiminase, type I	Hs00203458_m1
PADI4	peptidyl arginine deiminase, type IV	Hs00202612_m1
PCNA	proliferating cell nuclear antigen	Hs00696862_m1
PECAM / CD31	platelet/endothelial cell adhesion	Hs00169777_m1
GADD34	growth arrest and DNA damage-inducible 34	Hs00169585_m1
PDRM1 / BLIMP1	PR domain containing 1, with ZNF domain	Hs00153357_m1
RAG1	recombination activating gene 1	Hs00822415_m1
RELA / NFκB-p65 subunit	v-rel reticuloendotheliosis homolog A (p65)	Hs01042010_m1
CD138 / syndecan 1	syndecan 1	Hs00896423_m1

Common name used in this paper / alternative name(s)	Description	Assay ID
syndecan 4	syndecan 4	Hs01120909_m1
SELL / CD62L	selectin L	Hs01046459_m1
SENP1	SUMO1/sentrin specific peptidase 1	Hs00205764_m1
SIRT1	sirtuin 1	Hs01009006_m1
SIRT7	sirtuin 7	Hs00213029_m1
STAT1	signal transducer and activator of transcription	Hs01014002_m1
STAT5A	signal transducer and activator of transcription 5A	Hs00559643_m1
STAT5B	signal transducer and activator of transcription 5B	Hs00273500_m1
SUMO1	SMT3 suppressor of mif two 3 homolog 1	Hs02339311_g1
SUMO4	SMT3 suppressor of mif two 3 homolog 4	Hs01940570_g1
SYVN1	synovial apoptosis inhibitor 1, synoviolin	Hs00381211_m1
TGFB1	transforming growth factor, beta 1	Hs00998133_m1
TNF	tumour necrosis factor-alpha	Hs99999043_m1
OPG / TNFRSF11B	Osteoprotegrin	Hs00900360_m1
TACI/CD26 TNFRSF13B	transmembrane activator and CAML interactor	Hs00963364_m1
BAFF-R / TNFRSF13C	BAFF-receptor	Hs00606874_g1
BCMA / TNFRSF17	B-cell maturation antigen receptor	Hs00171292_m1
TNFR1A	tumour necrosis factor receptor 1A	Hs01042313_m1
TNFR1B	tumour necrosis factor receptor 1B	Hs00961755_m1
TRAIL	TNF-Related Apoptosis Inducing Ligand	Hs00921974_m1
APRIL / TNFSF13	a Proliferation-Inducing Ligand	Hs00182565_m1
BAFF / BLYS TNFSF13B	B-cell activating factor/ B Lymphocyte Stimulator	Hs00198106_m1
TP53	tumour protein p53	Hs01034249_m1
VCAM/CD106	vascular cell adhesion molecule 1	Hs00365486_m1
VEGF	vascular endothelial growth factor A	Hs00900058_m1
XBP1	X-box binding protein 1	Hs00231936_m1
GADD45	growth arrest and DNA damage-inducible protein	Hs00169255_m1
GAPDH	glyceraldehyde-3-phosphate dehydrogenase	Hs99999905_m1
18s	eukaryotic 18S rRNA	Hs99999901_s1

The GAPDH (glyceraldehyde-3-phosphate dehydrogenase) gene was used as reference (assay: Hs99999905_m1).

Table S4. Synovitis, TA and IL-7 detection by IHC.

		Lining layer			Stromal layer		
		CD3 T-cells	CD20 B-cells	CD68 Macrophage	CD3 T-cells	CD20 B-cells	CD68 Macrophage
arVAS	rho, p	NS	NS	NS	0.649, <0.0001	0.484, 0.008	NS
SS	rho, p	NS	NS	NS	0.663, <0.0001	0.591, 0.001	NS
TA *	Diffuse (n=11)	0-3.77	0-2.1	3.7–51.3	0.24-3.65	0.47-9.3	1.3-30.7
	Aggregates (n=11)	0-3.6	0-1.8	7.5–52.2	0.54-21.96	0.4-13.63	0.84-46.85
	GCL (n=7)	0-3.97	0-3.34	5.35-90.91	4.43-31.83	2.06-47.7	0.97-32.75

SS synovitis score; *score range by IHC image analysis (% of + cells); NS: not significant.

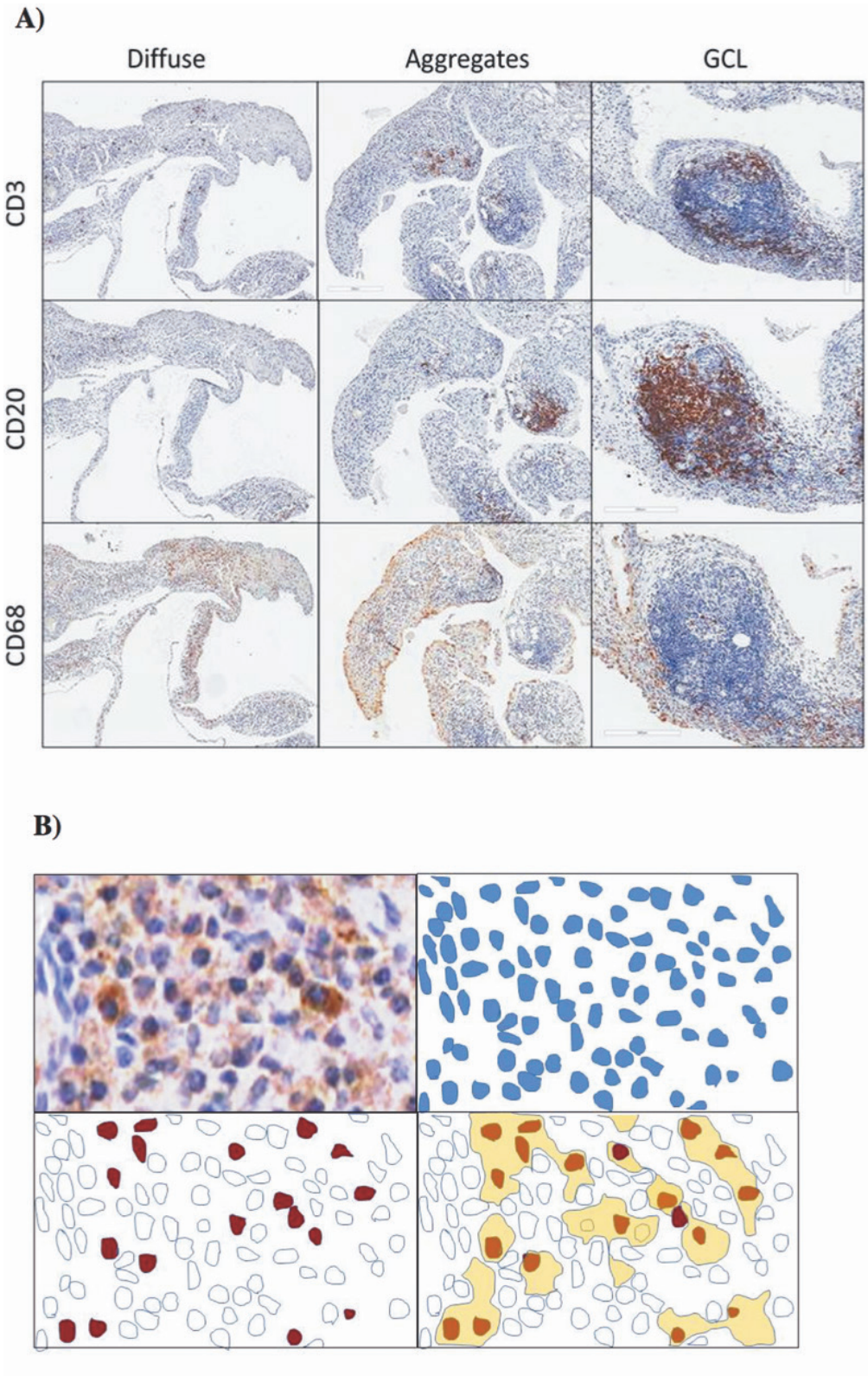

Fig. S1. A: Typical tissue architecture example with diffuse, aggregates or GCL structures, associated with lineage cell detection for T-cells (CD3), B-cells (CD20) and macrophages (CD68).
B: Scoring strategy using automated method and image capture by a multispectral Nuance camera connected to a microscope and operated using the Nuance software: top-left example of IHC results for IL7 staining, top right: cell all enumeration nucleus using haematoxylin (blue channel), bottom left : scoring for IL7+ nucleus (DAB+ brown channel over blue+ area), bottom right: scoring for total IL7 expression (nucleus and region of diffusion).

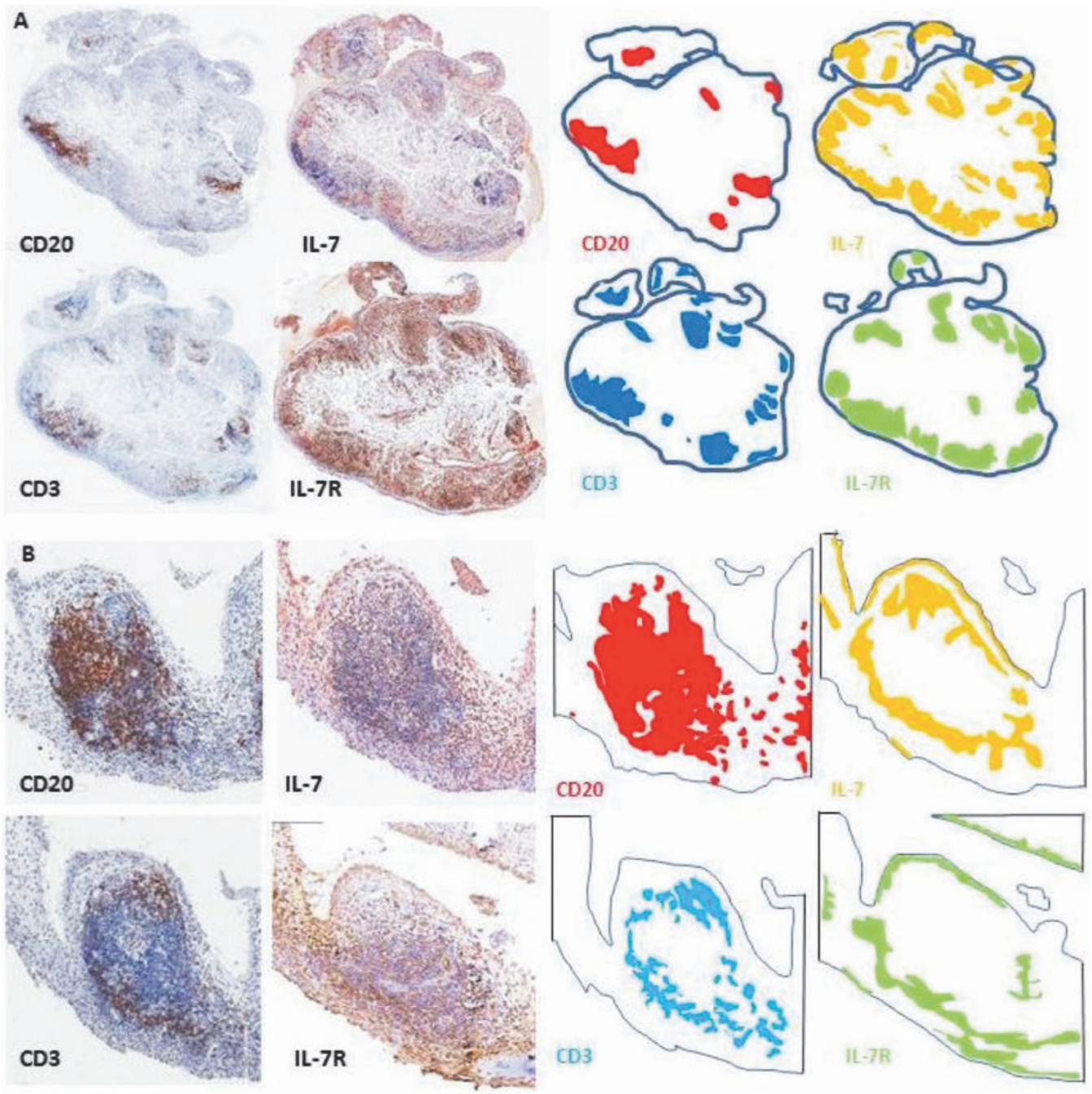

Fig. S2. Co-localisation of IL-7 and IL-7R expression relative to T (CD3) or B (CD20) cells. Left panels show IHC staining for each marker on consecutive sections of the tissue; Right panels shows image analysis of the staining based on DAB intensity.
A: biopsy containing separate aggregates of B and T-cells; **B**: biopsy containing GLC structures.