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Inflammasom-Aktivierung durch RNA-Viren und Pilze

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Für Mama, Papa und Katha

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Einleitung

1.1 Das Immunsystem und die Interaktion mit der Umgebung

Der Mensch steht zu jeder Zeit mit vielen anderen Organismen und Stoffen in seiner Umwelt in Kontakt. Verschiedene Nischen (z. B. Haut, Atmungswege, Verdauungstrakt) werden dabei von Mikroorganismen kolonisiert. Dies kann je nach Adaptationsstrategie des Mikroorganismus zum gegenseitigen Nutzen (z. B. im Kolon) oder zum Schaden des Wirts erfolgen[(1)]. Um eine Unterscheidung von nützlicher und schädlicher Besiedelung schnell und zuverlässig zu ermöglichen, haben Wirte im Laufe der Evolution verschiedene Mechanismen entwickelt, die die Interaktion mit Mikroorganismen steuern und verändern können. Grundlage ist dabei ein System zur Unterscheidung von «Fremd» und «Selbst», das eine Reihe von Abwehr- oder Toleranzmechanismen auslöst. Dieses Prinzip des Immunsystems existiert bereits in niederen Organismen wie Bakterien und hat sich in höheren Lebewesen komplex weiterentwickelt[(2)].

1.2 Das angeborene Immunsystem

Bereits bei primitiven Organismen existieren Abwehrstrategien, deren Effektormoleküle dauerhaft im Genom kodiert sind - das «angeborene» Immunsystem. Beim Menschen wird ein angeborenes Immunsystem zum einen durch passive Mechanismen wie physikalische (z. B. Epidermis) oder chemische (z. B. Muzine) Barrieren geschaffen, zum anderen gibt es Proteine und Zellen des angeborenen Immunsystems, die aktiv Fremdkörper oder eindringende Organismen bekämpfen[(1)]. Hierbei spielt die Erkennung von molekularen Mustern eine Rolle, die im Menschen nicht vorkommen, aber in Pathogenen evolutionär stark konserviert sind, weil es sich um für sie überlebensnotwendige Strukturen handelt (z.B. Lipopolysaccharide aus Zellwänden von gramnegativen Bakterien, bakterielles Flagellin oder β-Glucane aus Pilzen). Über solche *Pathogen-associated molecular-patterns* (PAMP) ist eine Unterscheidung von «Selbst» und «Fremd» möglich[(2)].
Im Menschen sind *Pattern-recognition-receptors* (PRR), die PAMP erkennen, ein wirkungsvolles Prinzip der angeborenen Abwehr. Die Aktivierung solcher Rezeptoren führt zur Initiation einer Vielzahl von Kaskaden des Immunsystems. Gleichzeitig existiert eine Vielzahl unterschiedlicher PRR: Sezernierte PRR binden Pathogene und führen so zur Aktivierung des Komplementsystems, das eine Opsonierung oder unspezifische Lyse auslöst[(1)].
Membrangebundene PRR können die Phagozytose von Pathogenen einleiten, welche in der Folge in Phagolysosomen zersetzt werden. Daneben gibt es PRR, die die Expression von Proteinen regulieren: die Bindung von Pathogenen oder PAMP führt zu einer intrazellulären Aktivierung von Signalwegen und zur Aktivierung von Transkriptionsfaktoren. Hierdurch kann neben einer direkten Aktivierung der erkennenden Zelle (z.B. Reifung oder Ausdifferenzierung) auch eine Sekretion von Chemo- und Zytokinen erfolgen, welche als Kommunikations- und Botenstoffe für andere Zellen dienen. Zellen des Immunsystems, die PRR exprimieren, sind also

eine erste Linie der Immunabwehr, indem sie andere Effektorzellen über die Präsenz von Pathogenen informieren können[1].

Ein Teil dieser Effektorzellen ist nach einer PRR-Stimulation für die Initiation einer inflammatorischen Reaktion verantwortlich. So werden beispielsweise über vasodilatierende Substanzen eine verstärkte Durchblutung und über die endotheliale Expression von Selektinen die extravasale Migration von neutrophilen Granulozyten angeregt[3]. Letztere degranulieren nach Kontakt mit Pathogenen oder durch Zytokinstimulation und schütten für Wirt wie Pathogen gleichermaßen toxische Substanzen aus[3],[4]. Nach der Elimination der Pathogene und des geschädigten Gewebes werden Wiederherstellungsprozesse eingeleitet, die auch über Zyto- und Chemokinsekretion gesteuert werden[3],[5].
Daneben existieren weitere Mechanismen der angeborenen Immunität wie das Interferon-System, die eine direkte Bekämpfung von Pathogenen ermöglichen[1],[6]. So aktivieren sezernierte Typ-I-Interferone NK-Zellen und steigern die Expression von antiviralen Proteinen in umgebenden Zellen - wodurch infizierte Zellen abgetötet und die Ausbreitung eines Virus verlangsamt werden kann[7].
Wie bei diesen lokalen, unspezifischen Antworten spielen Zytokine als Botenstoffe des Immunsystems auch für den gesamten Körper eine wichtige Rolle: eine Sekretion von IL-1β und IL-6 stimuliert beispielsweise im Hypothalamus eine Erhöhung der Körpersolltemperatur[8] oder im Knochenmark die Granulozytopoese[9] - was zu Fieber und Neutrophilie führt. Auch längerfristige Reaktionen wie Fibrosierung oder Gefäßneubildung werden durch proinflammatorische Zytokine wie IL-1β vermittelt[8].

Ursprünglich wurden v.a. Bestandteile von Mikroorganismen als PAMP postuliert, was eine Entzündungs- und Immunreaktion im sterilen Milieu (wie bei Gicht oder ischämischer Nekrose) aber nicht erklären konnte. Entsprechend existieren endogene Strukturen im Körper, die physiologischerweise nicht von PRR erkannt werden, aber unter bestimmten Umständen proinflammatorisch sein können[4],[10],[11]. Diese *Danger-associated-molecular-patterns* (DAMP) sind beispielsweise Harnsäure, ATP oder Kernproteine, die normalerweise durch Kompartimentierung von PRR getrennt sind. Bei unkontrolliertem Zelltod (z. B. durch Nekrose oder Infektion) treten diese Stoffe in den Extrazellulärraum und aktivieren dort PRR von Immunzellen[4].

Während DAMP und PAMP viele Gemeinsamkeiten wie teilweise gemeinsame PRR (siehe 1.5) haben, deutet der sehr unterschiedliche Verlauf von infektiösen und sterilen, durch DAMP ausgelösten Entzündungen darauf hin, dass gerade die Kombination und Integration verschiedener PRR-Signale über die weitere Kaskade entscheidet.

1.3 Das adaptive Immunsystem

Neben den keimbahnkodierten Rezeptoren entwickelte sich im Laufe der Evolution ein System, das eine wesentlich spezifischere Abwehr ermöglichte: durch die Rekombination von multiplen Genkassetten in ausdifferenzierten Zellen des Immunsystems wurde eine enorme Vielzahl an möglichen unterschiedlichen Gensequenzen geschaffen[12]. Die so kodierten T- und B-Zell- Rezeptoren (TCR bzw. BCR) erkennen durch diesen Mechanismus eine Vielzahl an Peptiden und molekularen Strukturen. Sie «adaptieren» sich an vorhandene Pathogene.

Reife T- und B-Zellen mit rekombinierter Rezeptorsequenz zirkulieren als langlebige Zellen in der Peripherie (T-Zellen werden zuvor im Thymus positiv und negativ selektiert, was Autoreaktivität und Anergie meist verhindern kann). Erst bei Bindung des entsprechenden Liganden am TCR bzw. BCR kommt es zu einer Aktivierung und klonalen Vermehrung. So sind lediglich wenige T- und B-Zellen in größerer Zahl im Körper aktiv sind, der Großteil ist ruhend bzw. naiv.

Über die TCR-vermittelte Erkennung präsentierter degradierter zellulärer Peptide auf *Major-histocompatibility-complex* (MHC)-I-Molekülen benachbarter Zellen können $CD8^+$-T Zellen gezielt infizierte, mutierte oder fremde Zellen erkennen und abtöten[(1)]. Von B-Zellen sezernierte spezifische Antikörper binden und aggregieren Strukturen, vereinfachen deren Phagozytose und verhindern mikrobielle Bewegung. Die Proliferation von B-Zellen und die Sekretion von Antikörpern erfolgt nach Kostimulation durch $CD4^+$-T-Zellen, wenn der TCR der $CD4^+$-T-Zelle den Antigenpeptid-MHC-II-Komplex der B-Zelle erkennt [(1)].
Neben diesen Hauptzelltypen gibt es noch eine Reihe weiterer Zelltypen (z. B. γδ-T-Zellen), auf die hier nicht näher eingegangen werden soll.

Diese «maßgeschneiderte» adaptive Immunantwort ist durch die definierten Liganden spezifischer auf eindringende Pathogene ausgerichtet als eine bloße Unterscheidung von «Fremd» und «Selbst» durch keimbahnkodierte Rezeptoren. Gleichzeitig ermöglicht die langzeitige Persistenz von bereits aktivierten Zellen die Ausbildung eines immunologischen Gedächtnisses durch *Memory*-Zellen.
Allerdings erfordert die große Anzahl an möglichen Antigenen die Präsenz von vielen ruhenden Zellen, deren Aktivierung genau dann und rasch auftreten soll, wenn die entsprechenden erkannten Strukturen tatsächlich auf Pathogenen präsent sind. Ein effektives adaptives Immunsystem wird bei Erstkontakt mit Pathogenen aber nur verzögert ausgebildet, und die Vielfalt an Mikroorganismen erfordert eine extrem hohe Anzahl an Zellen für eine optimale sensitive Erkennung von Peptiden pathogenen Ursprungs. Dies verdeutlicht, dass das adaptive System alleine nicht für eine Pathogenabwehr hinreichend sein kann.

1.4 Interaktion von angeborener und adaptiver Antwort

Eine Unterscheidung zwischen «Selbst» und «Fremd» ist durch das adaptive Immunsystem nur bedingt möglich (z. B. durch Negativselektion im Thymus) - Zellen mit bereits rekombinierter TCR- bzw. BCR-Sequenz wandeln sich bei Stimulation strikt in Effektorzellen und proliferieren, was bei entsprechenden Rezeptorsequenzen auch zu Autoimmunität führen kann. Dies verhindert jedoch die Interaktion von angeborener und adaptiver Immunität: die angeborene Antwort instruiert bei Erkennung von PAMP oder DAMP das adaptive System, welches durch orts- und zeitgleich vorliegende bzw. präsentierte Antigene aktiviert wird[(1)]. Über die weitere Ausprägung der Immunantwort entscheiden die jeweils aktivierten PRR. Die PRR-tragende APC fungiert hierbei als integrierende Instanz, indem sie entsprechend kostimulatorische Signale ausschüttet oder exprimiert und gegebenenfalls Peptide auf MHC-II-Molekülen präsentiert[(1), (13)].

Die dann gerichtete, adaptive T- und B-Zell-Antwort kann in Th1, Th2 und Th17 unterteilt werden[(14),(15),(16)]. Während die Th1-Antwort vor allem intrazelluläre Erreger bekämpft, ist eine Th2-Antwort gegen Infektionen mit mehrzelligen Parasiten und

Helminthen wirksam und führt bei Fehlaktivierung zu Allergien[15]. Für die wirksame Bekämpfung von Infektionen durch Pilze, Bakterien und Mykobakterien ist eine Th17-Antwort essentiell[15], [17], [18].
Entsprechend der Richtungen wurden auch Zytokin- und Effektorzellmuster definiert, die mit einer entsprechenden Aktivierung von B- und T-Zellen einhergehen[1]. IL-12 und IFN-γ werden als Th1-Zytokine beschrieben, während IL-4 und IL-5 eine Th2-Antwort einleiten[1]. Die genaue Entstehung der Th17-Antwort ist noch nicht ausreichend geklärt. IL-6, IL-17, IL-23, TGF-β und IL-1β scheinen eine wichtige Rolle zu spielen[17]. Als Effektorzellen rekrutiert eine Th1-Antwort vermehrt Makrophagen, wogegen eine Th2-Antwort Mastzellen und eosinophile Granulozyten und eine Th17-Antwort neutrophile Granulozyten rekrutiert. [15]

Für das Verständnis des Immunsystems ist es daher erforderlich, die verschiedenen PRR, deren Liganden und die Folgen der Stimulation in vitro wie in vivo zu kennen. Erst wenn die Initiation und Regulierung der B- und T-Zell-Antwort durch das angeborene Immunsystem verstanden ist, kann deren Potential auch therapeutisch bei Infektionen oder Tumoren sicher ausgenutzt werden.

1.5 Klassen der Pattern Recognition Receptors

1.5.1 Toll-like-Rezeptoren

Eine evolutionär konservative PRR-Gruppe sind die *Toll-like*-Rezeptoren (TLR), die von APC exprimiert werden. Die 10 (humanen) bzw. 12 (murinen) Rezeptoren erkennen, z.T. im Komplex, jeweils verschiedene Liganden (siehe Tabelle 1.1) und sind so für die Erkennung unterschiedlicher Pathogene verantwortlich[19].
Nach der Bindung der Liganden werden die Adapterproteine TRIF bzw. MyD88 aktiviert, was zu der nukleären Translokation der Transkriptionsfaktoren NFκB, der MAP Kinasen, IRF7 oder IRF3 führt. Daraufhin werden proinflammatorische Zytokine (nach NFκB- und MAPK-Aktivierung) und Interferone (nach IRF-Aktivierung) sezerniert, die die weitere Immunantwort steuern (meist in Richtung Th1 über die Sekretion von IL12p70[13], [20], [21]).

TLR	Adapterprotein	Ligand	Besonderheit
TLR1	MyD88	Triacyl Lipoprotein	als Dimer mit TLR2
TLR2	MyD88	Lipoprotein	
TLR3	TRIF	Doppelstrang(ds)-RNA	
TLR4	MyD88+TRIF	Lipopolysaccharid	
TLR5	MyD88	Flagellin	
TLR6	MyD88	Diacyl Lipoprotein	
TLR7	MyD88	Einzelstrang(ss)-RNA	aktiviert IRF7
TLR8	MyD88	ssRNA	aktiviert IRF7, in der Maus inaktiv
TLR9	MyD88	CpG-DNA	aktiviert IRF7
TLR10	MyD88	unbekannt	
TLR11	MyD88	*Profilin-like-molecule*	

Tabelle: Übersicht über TLR

1.5.2 C-type-Lektine

Eine weitere, sehr heterogene Klasse der PRR sind die *C-type*-Lektine (CLEC). Es existieren sezernierte, komplementaktivierende CLEC (z.B. Mannose-bindendes Lektin, MBL) und membranständige CLEC mit intrazellulärer Signaltransduktionsdomäne[22], die dann eine Phagozytose initiieren oder intrazelluläre Signalkaskaden aktivieren. Vor allem letztere scheinen für die Steuerung der Immunität von Bedeutung zu sein[22, 23].
Ein CLEC mit Signaltransduktionsfunktion ist Dectin-1 (CLEC7A), das für die Ausbildung einer Immunantwort gegen Pilze essentiell ist. Der natürliche Ligand ist β-Glucan, ein Zuckermolekül aus der Zellwand von Pilzen. Intrazellulär löst Dectin-1 über die Tyrosinkinase Syk eine Rekrutierung des Card9-Bcl10-Malt-1-Komplexes aus, was zur Aktivierung von NFκB führt[24]. Eine Stimulation von Dectin-1 induziert die Sekretion von IL-23, was zur Ausbildung einer Th17-Antwort führt[17]. Im Menschen sind neben Dectin-1 vermutlich noch weitere CLEC für solch eine systemische Immunantwort gegen Pilze verantwortlich[25].
Weitere Signaltransduktions-CLEC sind Dectin-2 (CLEC6A) und Mincle (CLEC4E), die eine wichtige Funktion in der Immunität gegen Pilze[26, 27] und Mykobakterien[18, 28] innehaben. Dectin-2 bindet verschiedene mannosehaltige Pilzwandbestandteile und induziert die Sekretion von Zytokinen, die für die Th17-Antwort bei einer Pilzinfektion essentiell sind[27]. Mincle bindet neben Pilzwandbestandteilen mit nachfolgender Zytokinproduktion[26] auch Glykolipid aus der Zellwand von Mycobacterium tuberculosis und trägt danach zur Granulombildung und Ausbildung einer Th1-Th17-Antwort bei[18, 28].
Außerdem wurden endogene CLEC-Liganden beschrieben, die bei nekrotischem Zelltod auftreten[23, 29]. Die resultierende Modulation des adaptiven Immunsystems ist unklar.

1.5.3 RIG-like-Helikasen

Immunzellen können über membranständige Rezeptoren effektiv Pathogene erkennen. Viren, die bereits in das Zytosol von Zellen eingedrungen sind, sind für diese PRR nicht mehr zugänglich - sie können über die intrazellulären *RIG-like*-Helikasen (RLH) RIG-I, MDA-5 und LGP2 erkannt werden. RLH erkennen dabei Nukleinsäuren, die während der Replikation von Viren entstehen.

RIG-I erkennt dsRNA, die am 5'-Ende ein Triphosphat trägt (5'-3pRNA). Die Triphosphatgruppe offenbart den viralen Ursprung, da humane RNA meist ein 7-Methyl-Guanosin-Triphosphat-Cap oder ein Monophosphat trägt und nicht als dsRNA vorliegt[30, 31, 32].
Obwohl beschrieben wurde, dass MDA-5 lange dsRNA (>300bp) erkennt und für die Erkennung von Picornaviren essentiell ist (im Gegensatz zu RIG-I), sind die Struktureigenschaften eines MDA-5-Liganden bisher weitgehend unbekannt. Experimentell wird lange dsRNA (polyI:C) als MDA-5-Stimulanz in vitro und in vivo verwendet[33].
Sowohl RIG-I als auch MDA-5 aktivieren nach Ligandenbindung eine Signalkaskade, die über das Adapterprotein MAVS in einer Translokation von NFκB und IRF und einer Produktion von proinflammatorischen Zytokinen und Typ-I-Interferonen münden[19].

Die Bedeutung von LGP2 ist nicht eindeutig geklärt: LGP2 erkennt zwar dsRNA ohne 5'-Triphosphat[34), (35)], besitzt aber keine N-terminalen CARD-Domänen, die an MAVS binden könnten. In in vitro-Studien wird eine negative Regulationsfunktion der RIG-I-abhängigen Immunstimulation beschrieben[(36), (37)], in vivo zeigte sich jedoch sowohl eine negative[(38)] als auch eine positive[(39)] Regulation des RIG-I-Signalweges. In vivo sind RIG-I und MDA-5 für die angeborene Immunität gegen bestimmte, teils verschiedene Virusklassen verantwortlich und initiieren eine Produktion von Typ-I-Interferonen und proinflammatorischen Zytokinen[(33)].

Protein	Aufbau	Ligand
RIG-I	CARD CARD Helicase RD	5'-3pRNA
MDA-5	CARD CARD Helicase RD	lange dsRNA
LGP2	Helicase RD	dsRNA
MAVS	TM CARD	

Tabelle: Übersicht über RLH

Für die Initiation der adaptiven Immunantwort in einigen viralen Infektionsmodellen scheint die Zytokinproduktion über RLH und TLR in vivo jeweils redundant zu sein. Die Ausbildung einer B- und T-Zellantwort kann sowohl durch konventionelle DC (cDC) - mittels RLH - als auch durch plasmazytoide DC (pDC) - mittels TLR - erfolgen. In anderen viralen Infektionsmodellen scheint die TLR-Stimulation eine größere Rolle für die adaptive Antwort zu spielen als die RLH-Stimulation[(40)]. Insgesamt ist die Rolle der RLH in der Steuerung der adaptiven Antwort nicht endgültig geklärt.

1.5.4 NOD-like-Rezeptoren und das Inflammasom

Ähnlich der intrazellulären Erkennung von Viren durch RLH können andere Pathogene im intrazellulären Kompartiment durch die Gruppe der *NOD-like*-Rezeptoren (NLR) erkannt werden. Sie bestehen aus einer LRR-Sensordomäne, einer NACHT-Oligomerisierungsdomäne und einer Signaldomäne (z.B. eine PYD- oder CARD-Domäne)[(41)].
Je nach Sensordomäne können eine Vielzahl verschiedener intrazellulärer Signale erkannt werden, je nach Signaldomäne entsprechende Transduktionswege aktiviert werden, wodurch die angeborene und auch die adaptive Immunität gesteuert werden.

Protein	Aufbau	Ligand	Signaltransduktion
NOD1	CARD NBD	Peptidoglykane (ie-DAP)	NFκB
NOD2	CARD NBD	Muramyldipeptid	NFκB
IPAF	CARD NBD	Gramneg. Bakterien	(Asc-) Caspase-1
NLRP3	PYD NBD	ROS, Cathepsin B	Asc-Caspase-1

Tabelle: Übersicht über NLR

Eine bedeutende Untergruppe der NLR sind solche, die Caspase-1 aktivieren können (z. T. über den Adapter Asc). Hierbei wird eine molekulare Plattform geschaffen, an der sich Caspase-1 autokatalytisch spalten und damit aktivieren kann. Durch diese «Inflammasom» genannte Plattform kann inaktives, intrazelluläres pro-IL-1β und pro-IL-18 durch Caspase-1 in die jeweils aktiven Formen proteolysiert und sezerniert werden.
Die Vorläuferproteine müssen zuvor durch PRR-NFκB-Stimulation (z. B. durch TLR) produziert werden. Für eine Sekretion von aktivem IL-1β sind also zwei Signale bzw. PRR-Stimuli nötig - die Sekretion von IL-1β ist damit (im Vergleich zu anderen Zytokinen) sehr stark reguliert[41].
IL-1β ist seit langem als Schlüsselzytokin bekannt, das nach seiner Freisetzung wichtig für die lokale wie systemische Entzündungsreaktion (z. B. die Fieberinduktion) ist[8]. Auch die adaptive Antwort wird durch IL-1β und IL-18 gesteuert[8]. Die molekularen Mechanismen, die zu einer Aktivierung des Inflammasoms führen, sind aber erst seit wenigen Jahren bekannt[42], so dass die Funktionsweise und Bedeutung der jeweiligen Signalwege in vivo noch großteils unklar ist.

Das Protein Nlrp3 ist wohl der am meisten untersuchte Inflammasom-NLR. Verschiedenste Pathogene, aber auch avitale (z.B. kristalline) Molekülstrukturen aktivieren diesen Komplex[42, 43]. Der genaue aktivierende Mechanismus ist dabei nicht geklärt. Die Generierung von reaktiven Sauerstoffspezies[44], mitochondriale Schädigung[45, 46] oder die Destabilisierung von Phagolysosomen[47] scheinen eine wichtige Rolle zu spielen. Diese Signale führen zu einem Fluss von K^+-Ionen aus der Zelle[48], zur Formierung eines Nlrp3 Oligomers, der Bindung des Adapters Asc und als Endstrecke zur autokatalytischen Spaltung von Caspase-1. Durch diesen Signalweg können neben Pathogenen auch endogene Strukturen erkannt und so das Immunsystem aktiviert werden[41]. Auch ATP, das bei Zelltod freigesetzt wird, aktiviert Nlrp3 indem es an den membranständigen K^+-Kanal P2X7 bindet[49].
Entsprechend der Vielzahl der beschriebenen Nlrp3-Stimulatoren zeigten $Nlrp3^{-/-}$-Mäuse eine verminderte angeborene Immunabwehr in verschiedensten Tiermodellen (u.a. Infektionen,[50] [51] [52] degenerative entzündliche Erkrankungen[53]). Auch die adaptive T-Zell-Antwort ist nach Immunstimulation in einigen Tiermodellen in $Nlrp3^{-/-}$-Mäusen defizient[54] [55] [56].
Neben diesem Nlrp3-Inflammasom bestehen weitere Proteinkomplexe, die durch andere Liganden stimuliert werden und in einer Aktivierung von Caspase-1 münden, z.B. Nlrp1 (Ligand Anthrax-Toxin), oder IPAF (Ligand gramnegative Bakterien)[41]. AIM2 ist ein Rezeptor für zytosolische dsDNA, der ein nicht-NLR-Inflammasom mit Asc bildet[57, 58].

Fragestellungen

2.1 RLH und das Inflammasom

Bei Infektionen durch RNA-Viren kommt es zu einer potenten Aktivierung von endosomalen (TLR3,7,8) und intrazellulären PRR (RLH) durch Nukleotide[1, 7]. Die Stimulation beider PRR-Klassen führt zur Produktion von Typ-I-Interferonen und proinflammatorischen Zytokinen[7, 59], die für die Ausbildung einer sofortigen angeborenen und z.T. auch einer späteren adaptiven Immunantwort notwendig sind.[40]

Bei RNA-Virusinfektionen wird außerdem Caspase-1 aktiviert und IL-1β sezerniert[60, 61]. Dessen Rezeptor IL1R1 ist in dabei in Tiermodellen für eine vollständige angeborene wie adaptive Immunantwort essentiell [62]. Die Mechanismen, welche PRR-Stimulation bei einer RNA-Virusinfektion eine Spaltung von Caspase-1 auslöst sind aber bisher unklar. Einige Studien postulieren eine Aktivierung des Nlrp3-Inflammasoms durch virale RNA[63, 64], was aber von anderen Gruppen nicht repoduziert werden konnte[48]. In einem Influenza-A-Modell war Nlrp3 zwar für die Produktion von IL-1β in vivo nötig, nicht aber für die adaptive Antwort und für das Überleben - im Gegensatz zu Asc, Caspase-1 und IL1R1[50]. Ähnliche Ergebnisse zeigten weitere Studien, in denen Influenza-A eine Nlrp3-abhängige angeborene Antwort auslöst [51, 52], die adaptive Antwort aber Nlrp3-unabhängig ist[51].
Es ist also unklar, durch welche Liganden und PRR verschiedene Inflammasome bei einer Virusinfektion aktiviert werden und wie diese Aktivierung zur Ausbildung einer adaptiven Antwort beiträgt.

Die vorliegende Arbeit behandelt diese Fragestellung vor allem unter dem Gesichtspunkt der Verknüpfung von RLH- und Inflammasom-Signalwegen. Für diesen Zweck wurde neben Viren als natürliche RLH-Liganden auch 5'-3pRNA als synthetischer Ligand von RIG-I verwendet, für den eine spezifische Aktivierung von RIG-I unter Umgehung der endosomalen TLR beschrieben ist[30]. Hierdurch sollten Artefakte durch den komplexen Prozess der Virusinfektion und eine etwaige Aktivierung anderer PRR vermieden werden können.

2.2 Inflammasom-Aktivierung bei Pilzinfektionen

Obwohl Pilze in der Umwelt des Menschen weit verbreitet sind, leiden immunkompetente Personen nur selten unter chronischen oder invasiven Infektionen. Dagegen sind immunsupprimierte Patienten besonders anfällig, da viele der Abwehrmechanismen des Körpers hier außer Kraft gesetzt sind. Bei AIDS-Patienten (depletierte $CD4^+$-T-Zellen) oder Tumorpatienten unter Chemotherapie (generelle Leukopenie) sind invasive Infektionen mit Sprosspilzen (z.B. *Candida albicans* oder *Cryptococcus neoformans*) häufig Todesursache[65].
Die genauen Mechanismen der Immunantwort gegen Sprosspilze sind nicht endgültig geklärt. Vor allem die initiale Steuerung der Immunantwort während einer systemischen Infektion - im Gegensatz zu einer oberflächlichen Besiedelung - erscheint unklar. Bei einer systemischen Infektion mit *C. albicans* kommt es neben der Initiation der angeborenen Antwort (Rekrutierung von Makrophagen und Neutrophilen, u.a. durch IL-6[66] auch zu einer Induktion einer adaptiven Immunreaktion in Th1- und Th17-Richtung. Die Stimulation der APC erfolgt dabei über TLR2- und TLR4-Liganden, was sowohl für eine direkte Elimination der Konidien als auch für die Ausbildung einer protektiven Th1-T-Zell-Antwort essentiell ist[67]. Neben dieser TLR-abhängigen Erkennung werden durch *C. albicans* auch Dectin-1-abhängige Mechanismen des angeborenen Systems aktiviert[68]. Diese Stimulation von APC führt zu einer Syk- und Card9-abhängigen Proliferation und Aktivierung von Th17-T-Zellen[17].

Mechanismen einer Inflammasom-Aktivierung durch Sprosspilze wurden bisher kaum beschrieben - allerdings ist bekannt, dass IL-1α, IL-1β[69] und IL-1RI[67] für eine kompetente Immunantwort in vivo unerlässlich sind. In vitro löst eine Stimulation mit *C. albicans* eine Produktion von IL-1β aus, was zu einer Produktion des Th1-polarisierenden Zytokins IFN-γ beiträgt[70]. Inwieweit verschiedene Inflammasom-Komponenten aber dann zu einer Steuerung der adaptiven Immunantwort beitragen, ist nicht hinreichend untersucht.

Bemerkenswert ist, das IL-1β im Mausmodell neben einer Hochregulation der Th1-Antwort auch zu einer Aktivierung von $Th17^+$-T-Zellen führen kann[71, 72]. Mäuse mit konstitutiv aktiven Inflammasom-Mutationen haben erhöhte IL-1β-Spiegel, eine überschießende Th17-Antwort sowie kutane entzündliche Infiltrate. Dieser Phänotyp ist durch Blockade des IL-1-Rezeptors oder von IL-17 reversibel[73]. Im Gegensatz hierzu leiden Patienten mit reduzierten Th17-Zytokinspiegeln[74] oder mit defektem Card9 und damit einhergehenden verminderten Th17-T-Zellzahlen[75] an chronischer mukokutaner Candidiasis. Patienten mit defektem STAT3, dem Adaptermolekül des IL-17- und des IL-22-Rezeptors, leiden am Hyper-IgE-Syndrom, was sich durch chronische kutane Infektionen manifestiert[76].

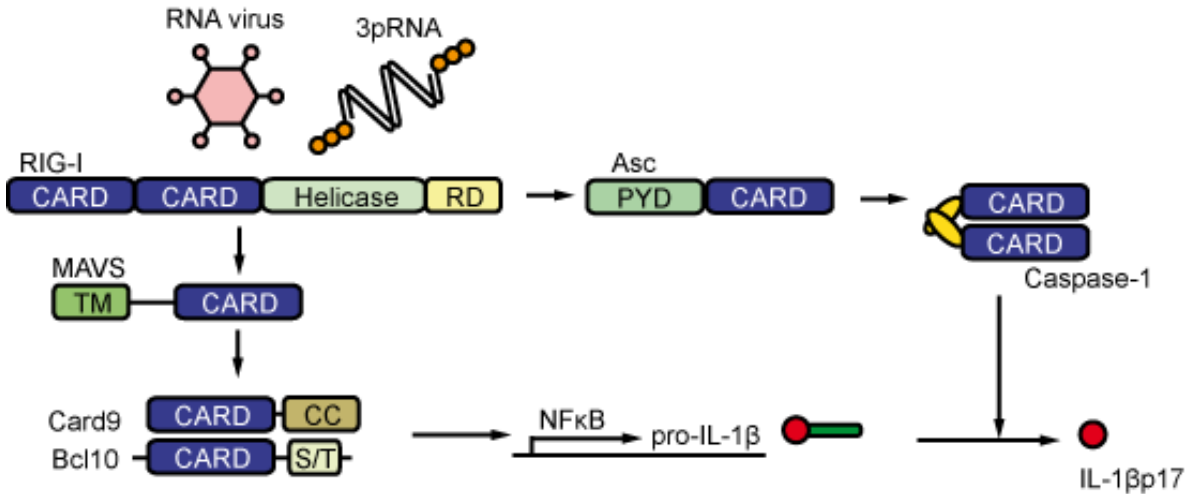
Die Regulation der Immunantwort durch IL-1β scheint also eine wichtige Rolle in der Kontrolle einer Infektion mit Sprosspilzen zu spielen - ein molekularer Mechanismus, der hierbei die Aktivierung des Inflammasoms und die Bildung von IL-1β erklärt, ist aber noch nicht gefunden.
Die vorliegende Arbeit behandelt diese Fragestellung mittels genetisch defizienter Mäuse und untersucht die Rolle von NLR und CLEC in der *C. albicans*-induzierten IL-1β-Sekretion.

Zusammenfassung der Originalarbeiten

3.1 Poeck et al.

In dieser Arbeit beschreiben wir eine doppelte Funktion von RIG-I in der virusinduzierten, proinflammatorischen Antwort. Während die RIG-I-vermittelte Aktivierung von NFκB das Adaptermolekül MAVS und einen Adapterkomplex aus CARD9 und Bcl-10 voraussetzt, bildet RIG-I auch einen Komplex mit dem Adapter Asc, um eine Caspase-1-abhängige Inflammasom-Aktivierung auszulösen. Diese ist unabhängig von MAVS, CARD9 und dem NLR-Protein Nlrp3. Im Gegensatz dazu führt eine Infektion mit MDA-5-aktivierenden Viren zu einer Aktivierung von NFκB und des Nlrp3-Asc-Inflammasoms.
Unsere Daten beschreiben den CARD9-Bcl-10-Komplex als eine notwendige Komponente der RIG-I-abhängigen proinflammatorischen Antwort und postulieren RIG-I als ein Sensormolekül, das nach Aktivierung direkt das Inflammasom aktivieren kann.

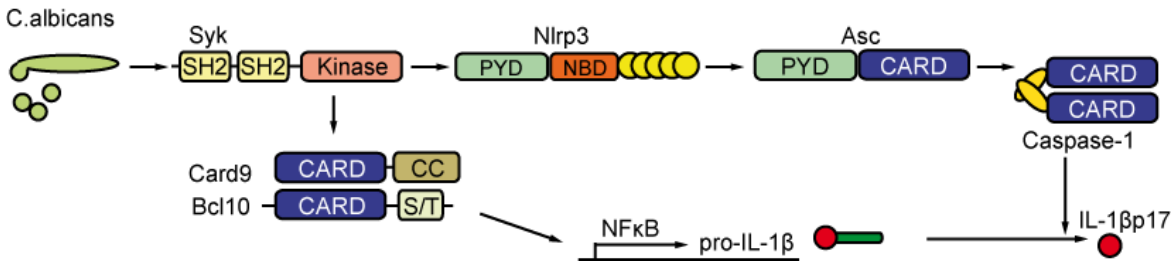
With this work we report a dual role for the RNA helicase RIG-I in RNA virus-induced proinflammatory responses. Whereas RIG-I-mediated activation of NFκB required the signaling adaptor MAVS and a complex of the adaptors CARD9 and Bcl-10, RIG-I also bound to the adaptor Asc to trigger caspase-1-dependent inflammasome activation by a mechanism independent of MAVS, CARD9 and the Nod-like receptor protein NLRP3. In contrast, infection with MDA-5-stimulating viruses leads to an activation of the Nlrp3-inflammasome.
Our results identify the CARD9-Bcl-10 module as an essential component of the RIG-I-dependent proinflammatory response and establish RIG-I as a sensor able to activate the inflammasome in response to certain RNA viruses.



3.2 Gross et al.

In dieser Arbeit zeigen wir dass die Tyrosinkinase Syk, die von mehreren ITAM-gekoppelten pilzerkennenden PRR aktiviert wird, sowohl die pro-IL-1β-Synthese als auch die Inflammasom-Aktivierung nach Stimulation mit *C. albicans* auslöst. Während der Syk-Signalweg für die pro-IL-1β Synthese Card9 benötigt, wird das Inflammasom durch die Generierung reaktiver Sauerstoffspezies und durch Kaliumefflux aktiviert. Genetische Deletion oder pharmakologische Inhibition von Syk verhindert spezifisch die Inflammasom-Aktivierung durch *C. albicans*, aber nicht durch andere Inflammasom-Aktivatoren wie *Salmonella typhimurium* oder das bakterielle Toxin Nigericin. Nlrp3 wurde als essentielles NLR-Protein identifiziert, das das Signal der Pilzerkennung an den Inflammasom-Adapter Asc weiterleitet und so eine Caspase-1-Aktivierung und pro-IL-1β-Spaltung auslöst. Entsprechend der Rolle des Nlrp3-Inflammasoms in der Immunantwort gegen Pilze zeigen wir, dass Nlrp3$^{-/-}$-Mäuse gegenüber einer Infektion mit *C. albicans* anfälliger sind als WT-Mäuse.
Unsere Ergebnisse erklären somit die molekulare Grundlage der IL-1β-Produktion bei Pilzinfektionen und identifizieren eine entscheidende Funktion des Nlrp3-Inflammasoms in der in vivo-Immunantwort.

Here we demonstrate that the tyrosine kinase Syk, operating downstream of several ITAM-coupled fungal pattern recognition receptors, controls both pro-IL-1β synthesis and inflammasome activation after cell stimulation with *C. albicans*. Whereas Syk signalling for pro-IL-1β synthesis selectively uses the Card9 pathway, inflammasome activation involves reactive oxygen species production and potassium efflux. Genetic deletion or pharmalogical inhibition of Syk selectively abrogated inflammasome activation by *C. albicans* but not by inflammasome activators such as *Salmonella typhimurium* or the bacterial toxin nigericin. Nlrp3 was identified as the critical NLR family member that transduces the fungal recognition signal to the inflammasome adaptor Asc for Caspase-1 activation and pro-IL-1β processing.
Consistent with an essential role for Nlrp3 inflammasomes in antifungal immunity, we show that Nlrp3$^{-/-}$ mice are hypersusceptible to *C. albicans* infection.
Thus, our results demonstrate the molecular basis for IL-1β production after fungal infection and identify a crucial function for the Nlrp3 inflammasome in mammalian host defence in vivo.



Originalarbeiten

4.1 Poeck et al.

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Recognition of RNA virus by RIG-I results in activation of CARD9 and inflammasome signaling for interleukin 1 beta production.

Poeck H*, Bscheider M*, Gross O*, Finger K, Roth S, Rebsamen M, Hannesschläger N, Schlee M, Rothenfusser S, Barchet W, Kato H, Akira S, Inoue S, Endres S, Peschel C, Hartmann G, Hornung V, Ruland J.
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Autorenbeteiligungen:
MB und HP waren in der Abteilung für Klinische Pharmakologie der Medizinischen Klinik Innenstadt an der LMU München (Abteilungsleiter: SE; Arbeitsgruppenleiter: GH, VH) tätig.
OG war in der Abteilung für Hämatologie und Onkologie der TU München (Abteilungsleiter: CP; Arbeitsgruppenleiter: JR) tätig.
Die Experimente wurden von MB, HP (in vitro- und in vivo-Experimente) und OG (in vivo-Experimente) durchgeführt, von HP und MB etabliert (u.a. in Kooperation mit MR am Institut für Biochemie der Universität Lausanne).
KF, SR, NH und MS waren an Versuchsdurchführungen beteiligt.
CP und SR waren an Diskussionen und Ergebnisinterpretation beteiligt.
WB, HK, SA und SI stellten Mäuse zur Verfügung.
HP, MB, OG, GH, VH und JR interpretierten die Ergebnisse und schrieben das Manuskript.

ARTICLES

nature immunology

Recognition of RNA virus by RIG-I results in activation of CARD9 and inflammasome signaling for interleukin 1β production

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Interleukin 1β (IL-1β) is a potent proinflammatory factor during viral infection. Its production is tightly controlled by transcription of *Il1b* dependent on the transcription factor NF-κB and subsequent processing of pro-IL-1β by an inflammasome. However, the sensors and mechanisms that facilitate RNA virus–induced production of IL-1β are not well defined. Here we report a dual role for the RNA helicase RIG-I in RNA virus–induced proinflammatory responses. Whereas RIG-I-mediated activation of NF-κB required the signaling adaptor MAVS and a complex of the adaptors CARD9 and Bcl-10, RIG-I also bound to the adaptor ASC to trigger caspase-1-dependent inflammasome activation by a mechanism independent of MAVS, CARD9 and the Nod-like receptor protein NLRP3. Our results identify the CARD9–Bcl-10 module as an essential component of the RIG-I-dependent proinflammatory response and establish RIG-I as a sensor able to activate the inflammasome in response to certain RNA viruses.

Viral infections are a constant threat to higher organisms, and early detection of viruses by the innate immune system is critical for host defense. Mammalian antiviral immunity is initiated by germline-encoded pattern-recognition receptors that recognize specific pathogen-associated molecular patterns such as viral nucleic acids. After recognizing viral RNA or DNA, pattern-recognition receptors activate signaling pathways that trigger the production of type I interferons and inflammatory cytokines to orchestrate immune responses for virus elimination and thereby produce the clinical symptoms of a viral infection[1]. The viral nucleic acid–recognition receptors include the transmembrane Toll-like receptors (TLRs) TLR3, TLR7, TLR8 and TLR9 (refs. 2–4), the HIN200 family member AIM2 (refs. 5–8) and the cytoplasmic RIG-I-like helicases (RLHs) RIG-I (encoded by *Ddx58*) and Mda5 (encoded by *Ifih1*)[9,10].

RLHs are responsible for the detection of viral RNA in the cytosol[1]. They are composed of an RNA-binding helicase domain, a regulatory domain and two caspase-recruitment domains (CARDs) for signal propagation to the interferon-regulatory factor (IRF) and transcription factor NF-κB signaling pathways. Despite such similarities, the RLHs RIG-I and Mda5 detect distinct RNA viruses. The viruses recognized by RIG-I include vesicular stomatitis virus (VSV) and influenza virus, whereas Mda5 controls responses to picornaviruses (encephalomyocarditis virus (EMCV) and poliovirus) and other viruses[11]. The selective ligand for RIG-I is a 5′ triphosphate on double-stranded RNA[12–15]. The natural ligand for Mda5 remains to be identified, but long stretches of polyinosinic-polycytidylic acid (poly(I:C)) can serve as an artificial agonist for this RLH[16].

To engage downstream pathways after recognizing a virus, RLHs form homotypic CARD-CARD interactions with the adaptor protein MAVS[17–19], which results in the recruitment and activation of further signaling molecules to mitochondria-associated complexes. The adaptors TRAF3, TANK and TRADD and the kinases TBK1 and IKKε are responsible for activation of the transcription factors IRF3 and IRF7 and subsequent synthesis of type I interferon[20]. RLHs additionally activate the proinflammatory NF-κB pathway for the production of cytokines such as interleukin 1β (IL-1β) and IL-6 (ref. 1), but the mechanisms that relay RLH signaling to NF-κB are not well defined.

The production of IL-1β requires, in addition to NF-κB-dependent new synthesis of pro-IL-1β, a second signal that triggers caspase-1 activation. Caspase-1 is responsible for the proteolytic processing of pro-IL-1β into mature, bioactive IL-1β. The activation of caspase-1 in response to many distinct danger signals depends on cytoplasmic multiprotein complexes called inflammasomes, which assemble

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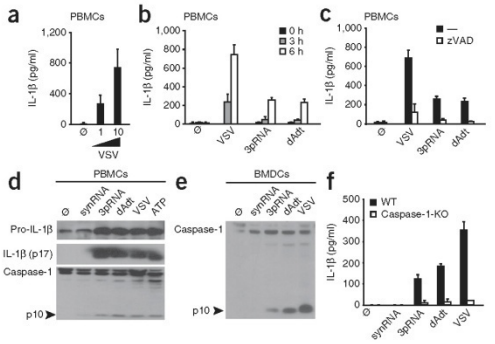

Figure 1 RIG-I signaling is required and sufficient for IL-1β production after infection with an RNA virus. (**a**,**b**) Enzyme-linked immunosorbent assay (ELISA) of IL-1β in supernatants of human PBMCs (2×10^6) left unstimulated (∅) or stimulated for 6 h with VSV at a multiplicity of infection of 1 or 10 (wedge; **a**) or for various times (key) with VSV (multiplicity of infection, 5) or 2 μg/ml of 3pRNA or poly(dA:dT) (dAdT; **b**). (**c**) ELISA of IL-1β production by PBMCs stimulated for 6 h as described in **b**, with (zVAD) or without (–) the addition of 0.05 μM z-VAD-fmk (pan-caspase inhibitor) 1 h before stimulation. (**d**) Immunoblot analysis of mature IL-1β (p17) and processed caspase-1 (p10 subunit) in supernatants of PBMCs stimulated with 2 μg/ml of synthetic dsRNA that lacks the 5′ triphosphate (synRNA), 3pRNA or poly(dA:dT), or with VSV (multiplicity of infection, 5). ATP (far right), LPS-primed PBMCs stimulated with 5 mM ATP (positive control). (**e**) Immunoblot analysis of caspase-1 processing (p10 subunit) in supernatants of BMDCs (1×10^6 cells per ml) stimulated for 6 h as described in **d**. (**f**) ELISA of IL-1β secretion by wild-type (WT) and caspase-1-deficient (Caspase-1-KO) BMDCs treated for 6 h with various stimuli (horizontal axis). Data are representative of three (**a**–**c**) or two (**f**) independent experiments (mean and s.e.m.) or are from one experiment representative of three (**d**) or at least three (**e**) experiments.

from various sensors and associated adaptor proteins in a context-dependent manner[21]. The best understood of these is the NLRP3 (also called NALP3) inflammasome, which activates caspase-1 indirectly via the inflammasome adaptor ASC (also called Pycard) in response to very diverse triggers, including crystals (such as uric acid, silica and asbestos), bacterial pore-forming toxins (such as nigericin), vaccine adjuvants, fungi, and certain DNA and RNA viruses[21–25]. However, the molecular interactions that engage the relatively non-specific NLRP3 inflammasome in response to such distinct stimuli are unclear at present. Another type of inflammasome that has been linked to viral recognition is the AIM2 inflammasome[5–8]. AIM2 is a cytoplasmic DNA receptor that directly interacts with ASC to trigger caspase-1 activation and subsequent IL-1β secretion after infection with a DNA virus. Here we demonstrate that RIG-I serves as a dual sensor that can trigger both NF-κB-dependent production of pro-IL-1β and inflammasome activation in response to certain RNA viruses. In this context, RIG-I engages the CARD9–Bcl-10 module for NF-κB activation and triggers ASC for inflammasome activation in an NLRP3-independent manner.

RESULTS

RIG-I in IL-1β production after VSV infection

To investigate the functions of RIG-I in RNA virus–induced production of IL-1β, we first infected human peripheral blood mononuclear cells (PBMCs) with VSV (**Fig. 1**). PBMCs exposed to VSV secreted mature IL-1β in a dose- and time-dependent manner (**Fig. 1a**,**b**). To selectively assess the role of RIG-I triggering in IL-1β production without considering the effects of viral RNA on other receptor systems, including TLRs, we transfected the cells with the selective RIG-I agonist 5′-triphosphate RNA (3pRNA). RIG-I ligation was sufficient to induce IL-1β production similar to that induced by transfected double-stranded DNA (poly(dA:dT)), which activates the AIM2 inflammasome[5–8] (**Fig. 1b**). Different 3pRNA species with distinct sequences resulted in similar IL-1β secretion (**Supplementary Fig. 1**), which indicated that the specific RNA sequence was not involved.

Next we pretreated PBMCs with the pan-caspase inhibitor z-VAD-fmk (**Fig. 1c**). Caspase inhibition abrogated IL-1β production after stimulation with 3pRNA and poly(dA:dT) and resulted in much less secretion of IL-1β induced by VSV (**Fig. 1c**). Then we analyzed the autocatalytic formation of the active caspase-1 subunit p10 (**Fig. 1d**). Consistent with the production of the mature (p17) form of IL-1β, caspase-1 was processed after activation of the NLRP3 inflammasome induced by ATP after priming with lipopolysaccharide (LPS) or after stimulation of cells with 3pRNA, poly(dA:dT) or VSV (**Fig. 1d**). In contrast, stimulation of cells with a synthetic double-stranded RNA (dsRNA) that lacks the 5′ triphosphate and triggers TLR7 but not RIG-I (ref. 26) did not induce IL-1β production or caspase-1 activation (**Fig. 1d**). Transfection of 3pRNA or poly(dA:dT) or infection with VSV also induced robust caspase-1 activation and IL-1β production in mouse bone marrow–derived dendritic cells (BMDCs), but transfection of the synthetic dsRNA lacking the 5′ triphosphate did not (**Fig. 1e**). As IL-1β production in response to these stimuli was defective in BMDCs from caspase-1-deficient mice (**Fig. 1f**), we conclude that caspase-1 activation is required for the 3pRNA- or VSV-induced proinflammatory responses. Together these results demonstrate that RIG-I engagement activates caspase-1 for IL-1β production.

To investigate whether RIG-I signaling is required for IL-1β production, we used DCs from RIG-I-deficient mice[16] (**Fig. 2**). Consistent with published data indicating that RIG-I ligation

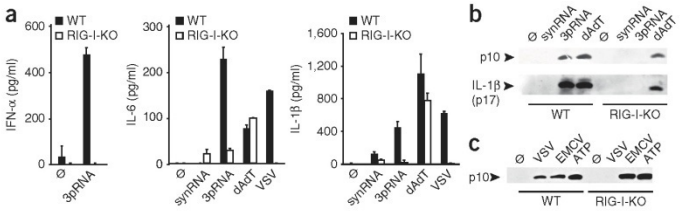

Figure 2 RIG-I controls IL-1β production and caspase-1 activation after detecting RNA viruses. (**a**) ELISA of IFN-α, IL-6 and IL-1β in supernatants of wild-type and RIG-I-deficient (RIG-I-KO) BMDCs treated for 6 h with various stimuli (horizontal axes). (**b**) Immunoblot analysis of caspase-1 processing (p10) and mature IL-1β (p17) in supernatants of the cells in **a**. (**c**) Immunoblot analysis of caspase-1 processing (p10) in wild-type and RIG-I-deficient BMDCs cells treated for 6 h with various stimuli (above lanes). ATP, LPS-primed BMDCs stimulated with 5 mM ATP (positive control). Data are representative of three independent experiments (mean and s.e.m.; **a**) or are from one experiment representative of three (**b**,**c**).

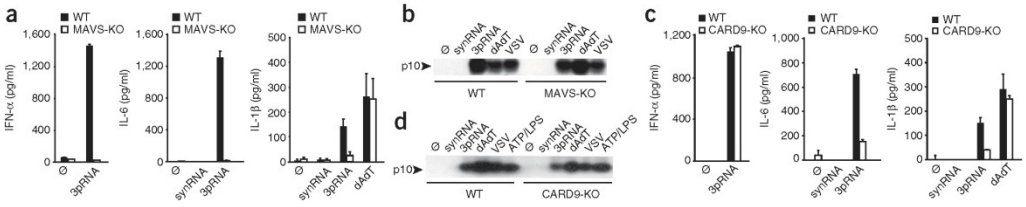

Figure 3 MAVS and CARD9 are essential for RIG-I-induced production of IL-1β but are dispensable for inflammasome activation. (**a**,**b**) ELISA of IFN-α, IL-6 and IL-1β in supernatants of wild-type and MAVS-deficient (MAVS-KO) BMDCs treated for 6 h with various stimuli (horizontal axes). (**b**) Immunoblot analysis of caspase-1 processing (p10) in supernatants of the cells in **a**. (**c**) ELISA of IFN-α, IL-6 and IL-1β in supernatants of wild-type and CARD9-deficient (CARD9-KO) BMDCs treated as described in **a**. (**d**) Immunoblot analysis of caspase-1 processing (p10) in supernatants of the cells in **c**. Data are representative of three (**a**) or at least four (**c**) independent experiments (mean and s.e.m.) or are from one experiment representative of three (**b**) or at least four (**d**) independent experiments.

activates the IRF and NF-κB transcription factors[10], RIG-I-deficient DCs were defective in IRF-controlled production of interferon-α (IFN-α) as well as in NF-κB-regulated secretion of IL-6 after stimulation with 3pRNA or VSV (**Fig. 2a**). RIG-I was required for VSV- or 3pRNA-triggered activation of caspase-1 and production of mature IL-1β p17, although poly(dA:dT) stimulation, exposure to EMCV or activation of NLRP3 with ATP and LPS induced normal activation of caspase-1 in RIG-I-deficient cells (**Fig. 2b**,**c**). Thus, RIG-I controls IRF- and NF-κB-dependent cytokine synthesis as well as inflammasome activation in response to certain RNA viruses.

RIG-I engages MAVS, CARD9 and Bcl-10

To understand the mechanisms that link RIG-I ligation to NF-κB-dependent synthesis of pro-IL-1β and to the activation of caspase-1 and inflammasomes, we investigated the role of the RIG-I effector MAVS in these pathways. Consistent with the essential role of MAVS in the activation of IRF and NF-κB[18,19], MAVS-deficient cells did not synthesize IFN-α or IL-6 after treatment with the RIG-I agonist 3pRNA (**Fig. 3a**). Moreover, MAVS-deficient cells did not secrete IL-1β after activation with RIG-I, although they produced normal amounts of IL-1β after stimulation with poly(dA:dT) (**Fig. 3a**). However, unlike RIG-I-deficient cells, MAVS-deficient BMDCs had normal activation of caspase-1 in response to stimulation with VSV or 3pRNA (**Fig. 3b**). These results indicate that although MAVS signaling is required for RIG-I-mediated production of IL-1β, MAVS engagement is not involved in RIG-I-mediated activation of caspase-1.

The E3 ubiquitin ligase TRIM25 catalyzes lysine 63–linked poly-ubiquitination of RIG-I to induce the recruitment of MAVS to RIG-I for the activation of effector pathways[27]. Consistent with the data reported above, TRIM25-deficient cells showed defects in IL-1β secretion after VSV infection (**Supplementary Fig. 2a**). However, caspase-1 was activated normally in VSV-infected or 3pRNA-stimulated TRIM25-deficient cells (**Supplementary Fig. 2b**).

Published work has identified the CARD coiled-coil protein CARD9 as a multifunctional adaptor that relays inputs from various pathogens to proinflammatory cascades[28]. We considered that CARD9 might also have a role in RIG-I signaling. To assess that possibility, we treated CARD9-deficient BMDCs with 3pRNA, synthetic dsRNA lacking the 5′ triphosphate, or poly(dA:dT). CARD9-deficient BMDCs showed much less of production IL-6 and IL-1β after stimulation with RIG-I, whereas the responses to poly(dA:dT) remained largely unchanged (**Fig. 3c**). Like MAVS, CARD9 was dispensable for caspase-1 activation (**Fig. 3d**). However, unlike MAVS, CARD9 was completely dispensable for IFN-α secretion (**Fig. 3c**). Thus, CARD9 selectively controls the RIG-I- and MAVS-induced proinflammatory response.

CARD9 is an upstream activator of the NF-κB pathway and of mitogen-activated protein kinases[29–31]. To define the role of CARD9 in RIG-I and MAVS signaling, we measured the activation of NF-κB in BMDCs stimulated with 3pRNA. RIG-I triggered a robust NF-κB response in wild-type BMDCs but not in cells lacking MAVS or CARD9 (**Fig. 4a**), which indicated that the two proteins act together to facilitate NF-κB activation. RIG-I engagement by 3pRNA or VSV also activated the kinases Jnk and p38, although in BMDCs this activation was independent of CARD9 (**Fig. 4b**). To determine the consequences of those findings for IL-1β production, we studied RIG-I-induced synthesis of pro-IL-1β. Consistent with the requirement for NF-κB activation in pro-IL-1β induction, both MAVS-deficient and CARD9-deficient BMDCs showed a defect in 3pRNA-induced synthesis of pro-IL-1β, although they responded normally to poly (dA:dT) (**Fig. 4c**). Accordingly, inhibition of canonical NF-κB signaling with a specific IKK kinase inhibitor, as well as CARD9 deletion, abrogated RIG-I-triggered upregulation of the secretion of pro-IL-1β mRNA and IL-1β protein after VSV infection (**Supplementary Fig. 3**). Thus, MAVS and CARD9 are essential for RIG-I-mediated activation of NF-κB and synthesis of pro-IL-1β. These findings explain why MAVS-deficient and CARD9-deficient cells fail to produce IL-1β although they regularly activate caspase-1 after RIG-I triggering.

CARD9-triggered activation of NF-κB depends on the CARD-containing adaptor Bcl-10 (refs. 28,30), which in turn can recruit the paracaspase MALT1 and use MALT1-dependent or MALT1-independent mechanisms for cell activation[32]. We next stimulated Bcl-10-deficient and MALT1-deficient cells with 3pRNA. BMDCs that lacked Bcl-10, like CARD9-deficient cells, showed severe defects in RIG-I-induced production of pro-IL-1β and secretion of IL-1β and IL-6 but had normal interferon responses (**Fig. 4d–f**) and caspase-1 activation (data not shown). The response to poly(dA:dT) remained unaffected by the Bcl-10 deletion (**Fig. 4e**,**f**). Notably, MALT1 was entirely dispensable for RIG-I-induced cytokine production (data not shown).

Together, the genetic experiments reported above demonstrated a signaling cascade downstream of RIG-I and MAVS that depended on CARD9 and Bcl-10 to control NF-κB-dependent cytokine production. To study the importance of this pathway *in vivo*, we infected wild-type and CARD9-deficient mice with VSV. Intravenous injection induced vigorous production of IFN-α, IL-6 and IL-1β in wild-type mice (**Fig. 4g**). Consistent with the *in vitro* results, the interferon responses were intact, but the concentrations of IL-6 and IL-1β were significantly lower in the serum of CARD9-deficient mice (**Fig. 4g**).

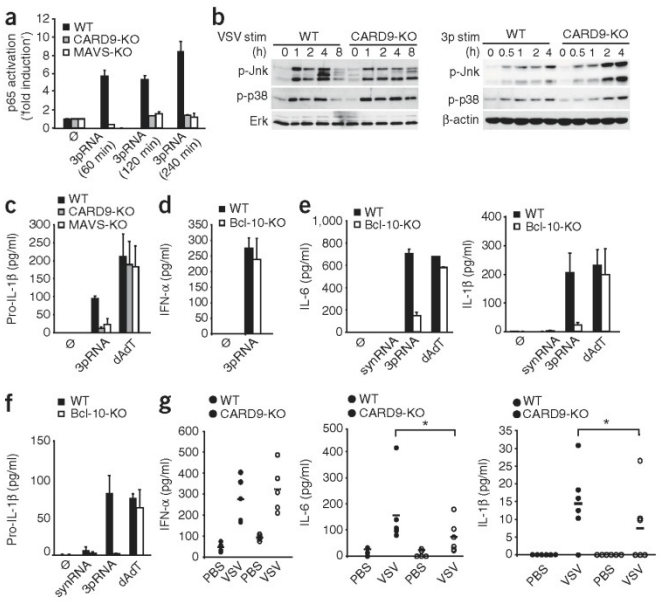

Figure 4 MAVS, CARD9 and Bcl-10 control pro-IL-1β production after RIG-I ligation. (**a**) Activation of the p65 subunit of NF-κB in nuclear protein extracts of wild-type, CARD9-deficient and MAVS-deficient BMDCs stimulated for 60, 120 or 240 min (horizontal axis) with 3pRNA, assessed by enhanced chemiluminescence and presented relative to activation in the unstimulated sample. (**b**) Immunoblot analysis of wild-type and CARD9-deficient BMDCs stimulated for various times (above lanes) with VSV (left) or 3pRNA (right), probed with antibodies specific for phosphorylated (p-) Jnk and p38. Bottom, immunoblot analysis of Erk and β-actin (loading control). (**c**) ELISA of intracellular pro-IL-1β in wild-type, CARD9-deficient and MAVS-deficient BMDCs stimulated for 6 h with 3pRNA or poly(dA:dT), assessed after cell lysis by repeated cycles of freezing and thawing. (**d–f**) ELISA of IFN-α (**d**), IL-6 and IL-1β (**e**), and intracellular pro-IL-1β (**f**) in supernatants of wild-type and Bcl-10-deficient (Bcl-10-KO) BMDCs treated with various stimuli (horizontal axes). (**g**) ELISA of IFN-α, IL-6 and IL-1β in serum collected from wild-type and CARD9-deficient mice 6 h after intravenous injection of 2×10^6 plaque-forming units of VSV or PBS (control). Each symbol represents an individual mouse; small horizontal lines indicate the mean. $*P < 0.05$ (two-tailed Student's *t*-test). Data are representative of at least three (**a**,**c**) or three (**d–f**) independent experiments (mean and s.e.m.) or two experiments (**g**) or are from one experiment representative of at least four independent experiments (**b**).

RIG-I and ASC form an NLRP3-independent inflammasome

As the role of the MAVS–CARD9–Bcl-10 axis in RIG-I-induced production of IL-1β is restricted to the control of pro-IL-1β synthesis, it is still unclear how RIG-I activates caspase-1. Because the cytosolic bacterium *Francisella tularensis* induces a type I interferon response to indirectly activate caspase-1 (ref. 33), we considered possible indirect effects of interferon in RIG-I-mediated inflammasome activation. However, *Irf3*$^{-/-}$*Irf7*$^{-/-}$ BMDCs[34] which do not produce type I interferons, had normal production of IL-1β after stimulation with 3pRNA or DNA (**Fig. 5a**).

Many classical triggers of inflammasomes require a stimulus-induced potassium efflux for caspase-1 activation[21]. We therefore assessed RIG-I-induced production of IL-1β before and after pre-incubation with the potassium channel inhibitor glibenclamide. This treatment completely abrogated 3pRNA- or VSV-induced secretion of IL-1β, whereas IL-6 production was unaffected (**Fig. 5b,c**). We obtained similar results by adding excess extracellular potassium (130 mM) to the medium before RIG-I stimulation (data not shown).

Danger sensors of the NLRP family and the DNA sensor AIM2 activate caspase-1 by binding to ASC[35]. To determine whether RIG-I and ASC also form a caspase-1-activating signaling complex, we immunoprecipitated endogenous ASC from THP-1 human monocytic cells before and after infection with VSV and studied potential RIG-I interactions by immunoblot analysis. RIG-I precipitated together with ASC in uninfected and VSV-infected cells, but Mda5 did not (**Fig. 5d**), which indicated that RIG-I and ASC can form a complex.

To investigate the function of RIG-I–ASC interactions by genetic means, we studied IL-1β production in BMDCs from ASC-deficient mice. In parallel, we analyzed wild-type and NLRP3-deficient DCs. Wild-type DCs showed robust caspase-1 activation and mature IL-1β production in response to triggering of RIG-I with 3pRNA or VSV,

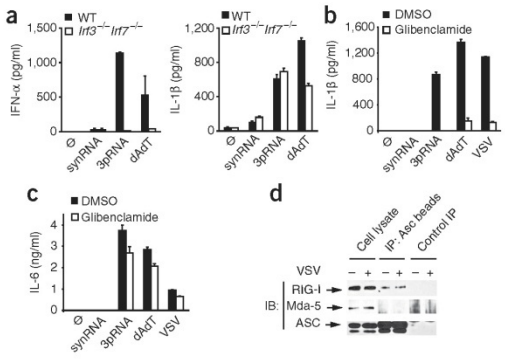

Figure 5 RIG-I engages ASC to induce inflammasome activation. (**a**) ELISA of IFN-α and IL-1β in supernatants of wild-type BMDCs and BMDCs doubly deficient in IRF3 and IRF-7 (*Irf3*$^{-/-}$*Irf7*$^{-/-}$) treated for 6 h with various stimuli (horizontal axes). (**b**,**c**) ELISA of IL-1β (**b**) and IL-6 (**c**) in supernatants of wild-type BMDCs treated for 6 h with various stimuli (horizontal axes) in the presence or absence (DMSO) of the potassium channel inhibitor glibenclamide. (**d**) Immunoassay of THP-1 cells infected with VSV (+) or left uninfected (–); immunoprecipitation of proteins from lysates with ASC-specific antibody (IP: Asc beads) or control antibody (Control IP) was followed by immunoblot analysis (IB) of immunoprecipitates (right four lanes) or total lysates (far left two lanes). Data are representative of two (**a**) or three (**b**,**c**) independent experiments (mean and s.e.m.) or are from one experiment representative of at least four independent experiments (**d**).

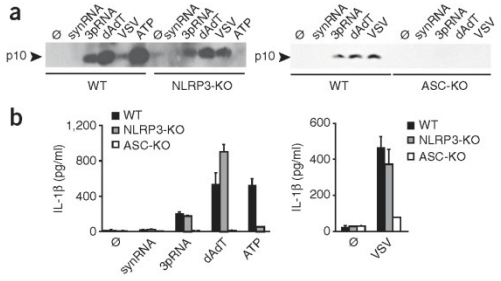

Figure 6 RIG-I-induced inflammasome activation is independent of NLRP3. (**a**) Immunoblot analysis of processed caspase-1 (p10) in supernatants of wild-type, NLRP3-deficient (NLRP3-KO) and ASC-deficient (ASC-KO) BMDCs treated with various stimuli (above lanes). ATP, cells pulsed with 5 mM ATP after 3 h of priming with ultrapure LPS (50 ng/ml). (**b**) ELISA of IL-1β in supernatants of the cells in **a**. Data are representative of at least three independent experiments (mean and s.e.m. in **b**).

after stimulation of AIM2 with poly(dA:dT) DNA, and after activation of NLRP3 with ATP after LPS priming (**Fig. 6a**). As expected, NLRP3-deficient DCs failed to activate caspase-1 (**Fig. 6a**) or to produce IL-1β (**Fig. 6b**) in response to ATP but responded normally to poly(dA:dT), as well as to triggering of RIG-I with 3pRNA or VSV. In contrast, ASC-deficient cells showed defective activation of caspase-1 and secretion of mature IL-1β after stimulation with ATP and LPS, poly(dA:dT), 3pRNA or VSV (**Fig. 6**). Thus, ASC is essential for coupling RIG-I-mediated recognition of RNA to NLRP3-independent caspase-1 activation for IL-1β production. We have proposed a model for the RIG-I-triggered proinflammatory responses and inflammasome activation (**Supplementary Fig. 4**).

Picornavirus engages Mda5, CARD9 and NLRP3

Finally, we investigated whether Mda5 engages similar mechanisms to induce IL-1β production. For this, we first infected Mda5-deficient or CARD9-deficient cells with the Mda5-engaging picornavirus EMCV[16]. Wild-type cells produced IL-1β robustly after EMCV infection (**Fig. 7a**,**b**). However, neither Mda5-deficient nor CARD9-deficient cells showed an IL-1β response (**Fig. 7b**), which indicates that CARD9 is an effector of Mda5 signaling. Further experiments demonstrated that CARD9 was required for EMCV-induced activation of NF-κB and was involved in pro-IL-1β synthesis (**Fig. 7c**,**d**). Next we assessed the role of Mda5 in inflammasome activation. In contrast to RIG-I activation in response to 3pRNA, transfection of poly(I:C) as an agonist for Mda5 (ref. 16) did not induce caspase-1 activation (**Fig. 7e**), which suggested that Mda5 activation by itself might not be sufficient for inflammasome activation. To study the requirement for Mda5 in inflammasome activation in response to the intact EMCV virus, we infected Mda5-deficient cells with EMCV (**Fig. 7f**). In parallel, we incubated the cells with the RIG-I-activating virus VSV and also infected NLRP3-deficient cells with the two viruses. Consistent with our results above (**Fig. 6a**,**b**), VSV strongly activated caspase-1 and led to potent IL-1β secretion in NLRP3-deficient cells. VSV also induced inflammasome activation in Mda5-deficient BMDCs (**Fig. 7f**). In contrast, EMCV infection required NLRP3 and Mda5 for caspase-1 activation and IL-1β production (**Fig. 7f–h**).

DISCUSSION

Here we have reported the identification of two RLH effector mechanisms that act together in the production of proinflammatory cytokines in response to recognition of RNA viruses. Although RLH-induced activation of NF-κB depended on the CARD9–Bcl-10 complex, RIG-I also activated the inflammasome by forming a signaling complex with ASC. Published work has demonstrated that RIG-I uses MAVS for activation of NF-κB and IRF[17–19]. We found that CARD9- and Bcl-10-deficient cells had profound defects in RIG-I-induced production of IL-6 and pro-IL-1β but had normal interferon responses, which provided genetic evidence that CARD9 and Bcl-10 act together downstream of MAVS to selectively control proinflammatory responses. Like RIG-I, Mda5 signals through MAVS[1]. We also observed a requirement for CARD9 in Mda5-induced production of proinflammatory cytokines. Thus, CARD9 represents a common and essential switch in RLH signaling that segregates the proinflammatory response from interferon production.

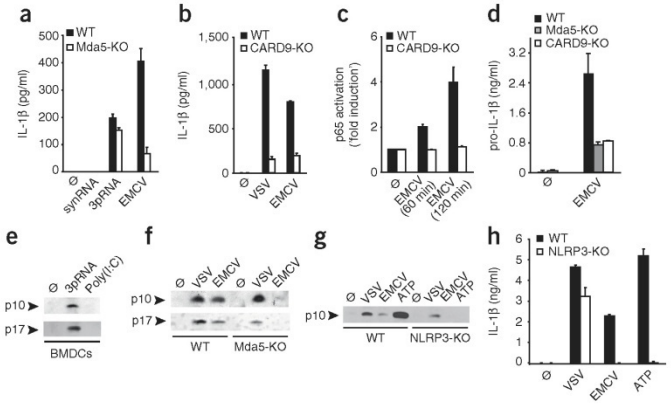

Figure 7 Mda5 requires NLRP3 for inflammasome activation. (**a**,**b**) ELISA of IL-1β in supernatants of wild-type, Mda5-deficient (Mda5-KO) and CARD9-deficient BMDCs treated for 6 h with various stimuli (horizontal axes). (**c**) Activation of the p65 subunit of NF-κB in nuclear protein extracts of wild-type and CARD9-deficient BMDCs stimulated for 60 or 120 min with EMCV, assessed by enhanced chemiluminescence and presented relative to activation in the unstimulated sample. (**d**) ELISA of intracellular pro-IL-1β in wild-type, Mda5-KO and CARD9-deficient cells, measured after cell lysis by repeated cycles of freezing and thawing. (**e**) Immunoblot analysis of caspase-1 processing (p10) and mature IL-1β (p17) in BMDCs stimulated for 6 h with 3pRNA or poly(I:C). (**f**) Immunoblot analysis of caspase-1 processing (p10) and mature IL-1β (p17) in wild-type and Mda5-deficient BMDCs infected for 6 h with VSV or EMCV. (**g**,**h**) Immunoblot analysis of caspase-1 processing (p10; **g**) and ELISA of IL-1β (**h**) in supernatants of wild-type and NLRP3-deficient BMDCs infected for 6 h with VSV or EMCV. ATP, LPS-primed BMDCs stimulated with 5 mM ATP (positive control). Data are representative of three (**a**,**b**,**d**), two (**c**) or at least three (**h**) independent experiments (mean and s.e.m.) or are from one experiment representative of QQ (**e**), three (**f**) or at least three (**g**) independent experiments.

CARD9 relays signals from various pattern-recognition receptors to proinflammatory pathways[28,36]. Together with its effector Bcl-10, CARD9 controls activation of the canonical NF-κB pathway after ligation of surface receptors containing or coupled to an immunoreceptor tyrosine-based activation motif, including dectin-1, FcγRIII, TREM-1 and Mincle[37]. This function of CARD9 is important for host defense against fungi and innate responses to certain bacteria, such as *Mycobacterium tuberculosis*[30,38]. Consistent with the function of CARD9–Bcl-10 complexes in NF-κB activation, we observed defective NF-κB signaling in CARD9-deficient and Bcl-10-deficient cells stimulated with agonists of RIG-I or Mda5. Those findings establish CARD9–Bcl-10 complexes as a missing link between RLHs and NF-κB. Previous work has shown that CARD9 can activate Jnk and p38, at least after macrophage infection with whole VSV particles[39]. However, we did not detect defects in Jnk or p38 signaling in CARD9-deficient DCs stimulated with 3pRNA. The fact that VSV triggers several innate signaling systems, including TLRs[40], and that CARD9 signaling to Jnk and p38 differs in DCs and macrophages[29] could explain why we did not observe Jnk or p38 signaling defects in our experiments, which focused specifically on the RLH-induced pathway.

Bcl-10 is also required for T cell and B cell antigen receptor signaling. In lymphocytes, Bcl-10 engages TRAF2 and TRAF6 to mediate lysine 63–linked polyubiquitinylation of IKKγ, which induces subsequent IKK activation[32]. It is therefore possible that a similar mechanism operates downstream of RLHs. Caspase-8 and FADD are also involved in antigen receptor–induced NF-κB activation[41], and a study has reported roles for caspase-8 and FADD downstream of MAVS[42]. Thus, several NF-κB activators seem to be shared by the RLH and antigen-receptor pathways.

Our work has additionally established RIG-I as a cytosolic RNA sensor for inflammasome activation. Selective RIG-I triggering was sufficient for caspase-1 activation and RIG-I was required for inflammasome activation in response to stimulation with 3pRNA or infection with VSV. In contrast to NF-κB signaling and pro-IL-1β production, this process was entirely independent of MAVS, TRIM25 and CARD9; it was also independent of NLRP3. To our knowledge, this represents the first example of MAVS-independent RIG-I signal transduction. RIG-I-induced inflammasome activation shows similarities to the activation of AIM2 inflammasomes by viral double-stranded DNA, which is also NLRP3 independent[35]. After binding double-stranded DNA, AIM2 interacts with ASC to induce ASC oligomerization and subsequent IL-1β production. Likewise, RIG-I can form a protein complex containing ASC, potentially with additional components, to induce IL-1β production in response to some RNA viruses. Thus, RIG-I and ASC can constitute a distinct inflammasome.

Influenza virus activates the NLRP3 inflammasome[22,24,25]. Influenza enters the cell via the lysosomal pathway, and lysosomal damage, which is a common NLRP3 activator, could trigger NLRP3 in this context. Consistent with that, lysosomal maturation is essential for influenza virus–induced NLRP3 activation[22]. We also observed a requirement for NLRP3 in IL-1β production after infection with EMCV. In contrast, VSV did not activate the NLRP3 inflammasome and instead relied on RIG-I and ASC. Our results and previously published data therefore indicate that inflammasome activation by RNA viruses can in principle use NLRP3-dependent or NLRP3-independent mechanisms. The precise viral ligands that trigger the NLRP3 inflammasome and the cellular sensors that detect these ligands remain to be identified. Although Mda5 was required for ECMV-induced inflammasome activation, ligation of Mda5 with poly(I:C) was not sufficient to induce inflammasome activation. In this context, the function of Mda5 might thus be restricted to priming of the NLRP3 inflammasome; that is, activation of Mda5 could potentially upregulate NLRP3, which is an essential step for activation of the NLRP3 inflammasome[43,44].

Together our findings have indicated that RLHs can trigger at least three different cellular responses: interferon production, NF-κB activation and inflammasome activation. RIG-I could in principle signal from one large signaling complex that 'fine tunes' interferon and proinflammatory responses. Alternatively, RIG-I might be a sensor in several distinct signalosomes: one may contain RIG-I together with MAVS, TRAF3 and TBK-1 for IRF activation; a second may involve MAVS, CARD and Bcl-10 for the activation of NF-κB; and a third might contain RIG-I together with ASC and potentially other factors for caspase-1 activation. Precisely how RIG-I integrates these cellular responses will be an important topic of future research, but our results offer one molecular explanation for the longstanding finding that RNA viruses are potent inducers of proinflammatory cytokines such as IL-1β and IL-6. In addition, as activation of the inflammatory responses forms a critical link to the induction of adaptive immunity, our results may have implications for the development of vaccines.

METHODS

Methods and any associated references are available in the online version of the paper at http://www.nature.com/natureimmunology/.

Note: Supplementary information is available on the Nature Immunology website.

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AUTHOR CONTRIBUTIONS

H.P., M.B., O.G., G.H., V.H. and J.R. designed the research; H.P., M.B., O.G., K.E., S.R., N.H., M.R. and M.S. did experiments; W.B., H.K., S.A. and S.I. contributed critical reagents; H.P., M.B., O.G., S.R., S.E., C.P., V.H., G.H. and J.R. analyzed results; H.P. and M.B. prepared the figures; and H.P., M.B., O.G., G.H. and J.R. wrote the paper.

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ONLINE METHODS

Mice. Mice genetically deficient in NLRP3, ASC, RIG-I, IRF3 and IRF7, CARD9, Bcl-10, MALT1, MAVS, Mda5, TRIM25 or caspase-1 have been described[16,27,30,34,45–48]. Mice were 6–12 weeks of age at the onset of experiments and were used according to local guidelines. Animal studies were approved by the local regulatory agency (Regierung von Oberbayern, Munich, Germany).

Media and reagents. RPMI-1640 medium (Invitrogen) and DMEM (Invitrogen) were supplemented with 10% (vol/vol) FCS (Hyclone), 3 mM L-glutamine, 100 U/ml of penicillin and 100 μg/ml of streptomycin (all from Sigma-Aldrich). ATP (A6419), poly(dA:dT) sodium salt (P9764), Glyburide (glibenclamide) and Bay11-7082 (specific IKK kinase inhibitor) were from Sigma-Aldrich. OptiMEM reduced-serum medium was from Invitrogen. Both poly(I:C) and ultrapure LPS (from *Escherichia coli* strain K12; used at a concentration of 50 ng/ml) were from Invivogen. The pan-caspase inhibitor z-VAD-fmk (benzyloxycarbonyl–Val-Ala-Asp–fluoromethylketone) was from Calbiochem. Chemically synthesized RNA oligonucleotides were from MWG-Biotech. Double-stranded *in vitro*–transcribed 3pRNA was generated as described[26]. Synthetic dsRNA lacking the 5′ triphosphate (sense, 5′-GCAUGCGACCUCUGUUUGA-3′) and 3pRNA1 (sense, 5′-GCAUGCGACCUCUGUUUGAC-3′) were used in most experiments; 3pRNA2 (sense, 5′-GCAUGCGAGGACUGUUUGAC-3′) was used to exclude sequence-specific effects on inflammasome activation.

Cells. Human PBMCs were isolated from whole human blood of healthy, voluntary donors by Ficoll-Hypaque density-gradient centrifugation (Biochrom). BMDCs were generated and grown as described[23]. Experiments involving human materials were in accordance with precepts established by the Helsinki Declaration and approved by the local ethics committee.

Cell culture and stimulation. All cells were stimulated in OptiMEM reduced-serum medium (Invitrogen) at a density of 1×10^6 cells per ml. Where not indicated otherwise, cells were incubated for 6–8 h with 2 μg/ml of synthetic RNA, 3pRNA or poly(dA:dT). Cells were transfected with RNA and DNA with Lipofectamine 2000 according to the manufacturer's protocol (Invitrogen). In some experiments, ultrapure LPS (50 ng/ml) and ATP (5 mM) were used as positive controls. For all conditions, cell-free supernatants were analyzed for cytokine secretion by ELISA or cells were lysed for immunoprecipitation and/or immunoblot analysis. Glibenclamide (25 μM) and z-VAD-fmk (0.05 μM) were used as described[23].

Cytokine measurement. Cell supernatants and serum were analyzed for cytokine secretion by ELISA (BD, R&D Systems or PBL Biomedical Laboratories). For analysis of intracellular pro-IL-1β, cells were lysed by repeated cycles of freezing and thawing in RPMI medium containing 10% (vol/vol) FCS, 3 mM L-glutamine, 100 U/ml of penicillin and 100 μg/ml of streptomycin and were analyzed by ELISA.

Immunoblot analysis. Precipitated media supernatants or cell extracts were analyzed by standard immunoblot techniques[49]. Primary antibodies were polyclonal goat antibody to mouse-IL-1β (anti–mouse IL-1β; BAF401; R&D Systems), polyclonal rabbit anti–human IL-1β (D116; Cell Signaling), monoclonal mouse anti-RIG-I (Alme-1; Alexis), polyclonal rabbit anti-Mda5 (AT113; Alexis), polyclonal rabbit anti-caspase-1 (sc-514 and sc-515; Santa Cruz), and polyclonal rabbit anti-Erk (9102), monoclonal rabbit antibody to phosphorylated p38 (12F8; 4631), polyclonal rabbit antibody to phosphorylated Jnk (9251) and polyclonal rabbit anti-β-actin (4967; all from Cell Signaling).

NF-κB activation. Nuclear extracts were prepared according to standard methods, and 1 μg nuclear protein was analyzed with a NF-κB p65 Transcription Factor Assay kit (Pierce) as described[50].

Coimmunoprecipitation. Endogenous RIG-I was immunoprecipitated from 5×10^6 THP-1 cells seeded in 10-cm dishes with or without 3 h of VSV stimulation (multiplicity of infection, 10). Cells were lysed in ice-cold lysis buffer (150 mM NaCl, 50 mM Tris pH 7.0, 1 mM EDTA, 1% (vol/vol) Nonidet P-40, 10% (vol/vol) glycerol, 1 mM Na_3VO_4, 1 mM phenylmethyl sulfonyl fluoride and a Protease Inhibitor Cocktail tablet (Roche)) and lysates were incubated overnight at 4 °C with 80 μl protein G Sepharose beads (GE Healthcare) and 2 μg antibody (polyclonal anti-ASC (AL177; Alexis Biochemicals) or polyclonal rabbit anti–Syrian hamster immunoglobulin G (307-005-003; Jackson ImmunoResearch)). Immunoprecipitates were analyzed by immunoblot.

RNA extraction and quantification. Total RNA was extracted from cells with a High Pure RNA Isolation kit as described by the manufacturer (Roche) and was analyzed by quantitative RT-PCR. RNA (1 μg) was reverse-transcribed with SuperScript II Reverse Transcriptase and oligo(dT) oligonucleotide according to the manufacturer's protocol (Invitrogen). The Universal ProbeLibrary and LightCycler 480 system (Roche) were used for quantitative PCR (primer sequences, **Supplementary Table 1**). Gene expression was calculated as a ratio of the expression of the gene of interest to that of *Hprt1* (encoding hypoxanthine guanine phosphoribosyl transferase) measured for the same sample.

Preparation of virus stock and plaque assay. Baby hamster kidney (BHK-21) cells were infected with VSV Indiana (Mudd-Summers strain) or EMCV (a gift from A. Krug) and cell culture supernatants were collected 20 h after infection. Virus yield in culture supernatants was determined by standard plaque assay. VSV and EMCV were used at a multiplicity of infection of 5–10.

***In vivo* viral infection.** For viral infection, mice were given intravenous injection of 2×10^6 plaque-forming units VSV per mouse in 200 μl medium or an equal amount of PBS (as a control). Serum was collected after 6 h. Cytokine concentrations were measured by ELISA.

Statistical analyses. The statistical significance of differences was determined by the paired two-tailed Student's *t*-test. Differences with a *P* value of less than 0.05 were considered statistically significant.

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LETTERS

Syk kinase signalling couples to the Nlrp3 inflammasome for anti-fungal host defence

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Fungal infections represent a serious threat, particularly in immunocompromised patients[1]. Interleukin-1β (IL-1β) is a key pro-inflammatory factor in innate antifungal immunity[2]. The mechanism by which the mammalian immune system regulates IL-1β production after fungal recognition is unclear. Two signals are generally required for IL-1β production: an NF-κB-dependent signal that induces the synthesis of pro-IL-1β (p35), and a second signal that triggers proteolytic pro-IL-1β processing to produce bioactive IL-1β (p17) via Caspase-1-containing multiprotein complexes called inflammasomes[3]. Here we demonstrate that the tyrosine kinase Syk, operating downstream of several immunoreceptor tyrosine-based activation motif (ITAM)-coupled fungal pattern recognition receptors, controls both pro-IL-1β synthesis and inflammasome activation after cell stimulation with *Candida albicans*. Whereas Syk signalling for pro-IL-1β synthesis selectively uses the Card9 pathway, inflammasome activation by the fungus involves reactive oxygen species production and potassium efflux. Genetic deletion or pharmalogical inhibition of Syk selectively abrogated inflammasome activation by *C. albicans* but not by inflammasome activators such as *Salmonella typhimurium* or the bacterial toxin nigericin. Nlrp3 (also known as NALP3) was identified as the critical NOD-like receptor family member that transduces the fungal recognition signal to the inflammasome adaptor Asc (Pycard) for Caspase-1 (Casp1) activation and pro-IL-1β processing. Consistent with an essential role for Nlrp3 inflammasomes in antifungal immunity, we show that Nlrp3-deficient mice are hypersusceptible to *Candida albicans* infection. Thus, our results demonstrate the molecular basis for IL-1β production after fungal infection and identify a crucial function for the Nlrp3 inflammasome in mammalian host defence *in vivo*.

IL-1β is a central orchestrator of immunity against various classes of pathogens, and a key trigger of inflammatory diseases. Its production is mediated by pro-Caspase-1-processing inflammasomes that contain danger sensors such as Nlrp proteins or Nlrc4 (also known as Ipaf) to connect upstream signals to Caspase-1 activation[3]. Although rapid progress has been made in identifying inflammasome components, the mechanisms upstream of inflammasome activation are not well understood.

Opportunistic fungi are clinically important pathogens[1] that cause life-threatening infections in immunocompromised individuals. However, immune responses to fungi are ill characterized, and whether and how fungi activate inflammasomes is unknown. We have co-incubated murine bone-marrow-derived dendritic cells (BMDCs) with *C. albicans* and measured the production of IL-1β. As a control, we activated inflammasomes with several well-characterized stimuli, including lipopolysaccharide (LPS) and ATP[4]. Live but not inactivated *C. albicans* induce a robust production of IL-1β (Fig. 1a and Supplementary Fig. 1a–d). Murine bone-marrow-derived macrophages (BMDMs), human peripheral blood monocytes (PBMCs) or THP-1 cells showed similar responses, excluding cell type or species-specific effects (Supplementary Fig. 1e–g and data not shown). Cell stimulation with the fungus directly activated Caspase-1, as detected by the appearance of the p10 cleavage product (Fig. 1d). Because Caspase-1-deficient BMDCs or BMDCs treated with the caspase inhibitor z-VAD-fmk have severe defects in IL-1β production after *C. albicans* stimulation, despite normal intracellular pro-IL-1β accumulation or secretion of the inflammasome-independent cytokine TNF-α (Fig. 1a–c and Supplementary Fig. 1h), we conclude that *C. albicans* induces an IL-1β-dependent inflammatory response mediated by Caspase-1.

Several ITAM-containing or ITAM-coupled C-type lectins, including dectin-1 (encoded by *Clec7a*), dectin-2 (*Clec4n*), Mincle (*Clec4e*) and potentially others, were recently identified as signalling fungal pattern recognition receptors that trigger pro-inflammatory cytokine responses[5,6]. Although there is redundancy at the receptor

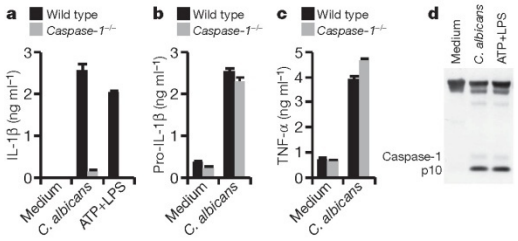

Figure 1 | *Candida albicans* activates a Caspase-1-dependent IL-1β response. a–c, BMDCs from wild-type or *Caspase-1*$^{-/-}$ mice were stimulated with *C. albicans* (5×10^6 cells ml^{-1}) for 5 h, or with ATP (5 mM) for 2 h after LPS prestimulation (0.5 ng ml^{-1} for 3 h). Secreted IL-1β (**a**), intracellular pro-IL-1β (**b**), or secreted TNF-α (**c**) were determined by ELISA. **d**, BMDCs were either left unstimulated (medium) or stimulated with *C. albicans* or with ATP after LPS priming. Caspase-1 activation was analysed by western blot, using an antibody against the Caspase-1 p10 cleavage product. Values in **a–c** are means and s.d. All results are representative of at least three independent experiments.

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level, Syk and its downstream adaptor Card9 are both essential for the production of cytokines such as TNF-α in response to fungal recognition[7–9]. To test whether these signal transducers are required for IL-1β production, we analysed responses in Syk-deficient BMDCs[9]. To exclude Syk-dependent effects on cell differentiation, we also inhibited Syk kinase activity in wild-type BMDCs with the specific small molecule Syk inhibitor R406 that is in clinical trials for inflammatory diseases[10,11]. Syk deletion or inhibition blocked intracellular pro-IL-1β accumulation as well as TNF-α production in response to *C. albicans* (Fig. 2a, b and Supplementary Fig. 2a, b), indicating that Syk activity controls the first signal for IL-1β production after fungal recognition.

Notably, we also found that Syk blockade inhibited Caspase-1 activation by *C. albicans*, although the bacterial toxin nigericin induced normal Caspase-1 processing in $Syk^{-/-}$ cells (Fig. 2c, d). Moreover, Syk deletion or inhibition blocked *C.-albicans*-induced IL-1β secretion, and the priming of $Syk^{-/-}$ or R406-treated cells with LPS, to ensure Syk-independent pro-IL-1β production, did not rescue these defects (Fig. 2e–g). In contrast, Syk blockade does not affect IL-1β production after treatment with ATP or nigericin that both activate Nlrp3 inflammasomes, or in response to *S. typhimurium* that activates Nlrc4 inflammasomes[12], excluding a general function for Syk in Caspase-1 activation (Fig. 2e, f, h and Supplementary Fig. 2c). Together, these data indicate that Syk kinase signalling controls both pro-IL-1β production and inflammasome activation specifically after fungal recognition. Because inflammasome activity is also required for pro-IL-18 processing[3,13], we also tested the role of Syk signalling in IL-18 secretion. In line with the data described earlier, LPS-primed $Syk^{-/-}$ cells do not secrete measurable amounts of IL-18 after stimulation with *C. albicans*, although they produce normal IL-18 levels in response to ATP or nigericin (Fig. 2i).

We next tested the role of Card9 in these pathways. $Card9^{-/-}$ BMDCs[7] also failed to produce IL-1β after *C. albicans* stimulation (Fig. 2j). Consistent with the requirement of Card9 in Syk-mediated NF-κB activation[7,8] pro-IL-1β synthesis was blocked in $Card9^{-/-}$ cells (Fig. 2k). Yet, in contrast to Syk inhibition, Card9 deletion did not effect *C.-albicans*-triggered Caspase-1 activation (Fig. 2l), and after LPS priming $Card9^{-/-}$ cells secreted mature IL-1β into the culture supernatant (Fig. 2l). Thus, the Card9 pathway transduces the Syk signal selectively to pro-IL-1β synthesis, but Card9 is dispensable for inflammasome activation. Toll-like receptor (TLR)-activated MyD88 signalling is also not essential for inflammasome activation by *C. albicans* (Supplementary Fig. 3a, b).

To determine the mechanisms by which *C. albicans* might activate Caspase-1, we considered the possibility that the fungus may indirectly trigger inflammasomes by inducing the release of ATP or other activators from dying cells[14]. However, *C. albicans* did not cause substantial cell injury (Supplementary Fig. 4a). By stimulating BMDCs from mice lacking the ATP receptor $P2X_7$ (also known as $P2RX_7$)[4], we additionally excluded a critical requirement for cellular ATP release in IL-1β production by *C. albicans* (Fig. 3a).

Most inflammasome activators trigger cellular intermediary signals that couple to the activation of NLR proteins[3]. Common mechanisms implicated in inflammasome activation include K^+ efflux[15], lysosomal damage with release and activation of cathepsin B[16,17], and reactive oxygen species (ROS) production[15,18,19]. To study the role of these signalling cascades in *C. albicans* inflammasome activation, we first blocked potassium channels with glibenclamide[20]. *Candida-albicans*-dependent IL-1β production was inhibited by blocking K^+ efflux although the secretion of TNF-α was largely unaffected (Fig. 3b, c). Next, the lysosomal cathepsin B pathway was blocked by inhibiting lysosomal acidification with bafilomycin A[17]. In parallel, we inhibited the NADPH-oxidase-dependent ROS system with (2*R*,4*R*)-4-aminopyrrolidine-2,4-dicarboxylate (APDC)[18] (Fig. 3d, e). Neither of these inhibitors affected *C.-albicans*-dependent TNF-α secretion (Fig. 3d). Bafilomycin A also did not influence IL-1β production,

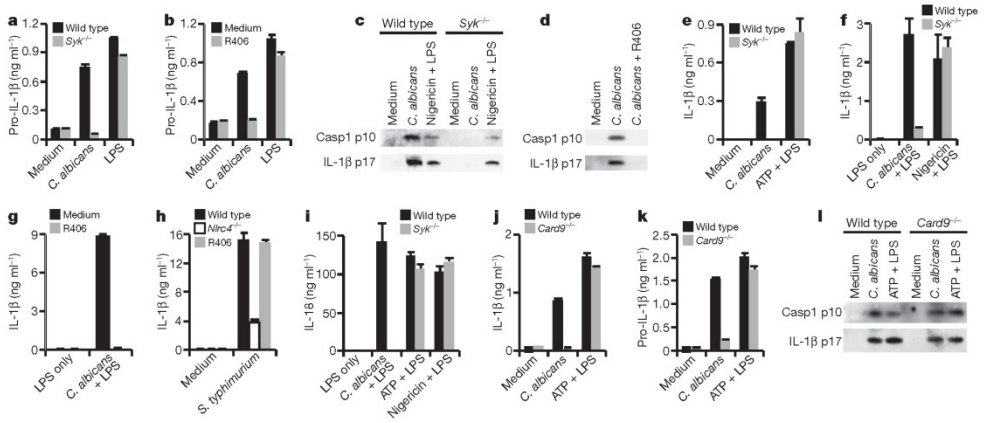

Figure 2 | Syk signalling controls pro-IL-1β synthesis and Caspase-1 activation after *C. albicans* stimulation. **a**, **b**, Wild-type or $Syk^{-/-}$ BMDCs, untreated or treated with 1 μM of the Syk inhibitor R406, were stimulated with *C. albicans* or LPS (100 ng ml^{-1}) before the measurement of intracellular pro-IL-1β. **c**, BMDCs from wild-type or $Syk^{-/-}$ mice were stimulated with *C. albicans* or with nigericin after LPS priming. Supernatants were analysed by western blot for the presence of Caspase-1 p10 and IL-1β p17 cleavage products. **d**, BMDCs were stimulated with *C. albicans*, with or without 1 μM R406 pretreatment. Processing of Caspase-1 and secretion of mature IL-1β p17 was analysed as in **c**. **e**, BMDCs from wild-type or $Syk^{-/-}$ mice were stimulated with *C. albicans* or with ATP and LPS before the measurement of IL-1β release. **f**, Wild-type or $Syk^{-/-}$ BMDCs were prestimulated with LPS and stimulated with *C. albicans* or nigericin before the measurement of IL-1β production. **g**, BMDCs were prestimulated with LPS, untreated or treated with R406 (30 min), and left unstimulated or stimulated with *C. albicans*. IL-1β production was measured by ELISA. **h**, BMDCs from wild-type mice, pretreated with R406 as indicated, or from $Nlrc4^{-/-}$ mice were stimulated with *S. typhimurium* (multiplicity of infection (m.o.i.) 10) for 5 h. IL-1β production was determined by ELISA. **i**, Cells were stimulated as in **e** and **f**, and IL-18 production was determined in the supernatants. **j**, **k**, BMDCs from wild-type or $Card9^{-/-}$ mice were stimulated with *C. albicans* or ATP and LPS. Secreted IL-1β (**j**) or intracellular pro-IL-1β (**k**) was determined as above. **l**, BMDCs from wild-type or $Card9^{-/-}$ mice were stimulated with *C. albicans* or ATP after LPS priming. Processing and secretion of Caspase-1 (p10) or IL-1β (p17) were determined by western blot. All values in **a**, **b** and **e**–**k** are means and s.d. Results are representative of at least three independent experiments.

suggesting that the lysosomal cathepsin B pathway is not involved in the *C. albicans* response (Fig. 3e). Consistently, BMDCs from cathepsin-B-deficient mice[21] also produce normal amounts of IL-1β after *C. albicans* exposure (Supplementary Fig. 4b). In contrast, ROS inhibition with APDC impaired *C.-albicans*-dependent IL-1β secretion in a dose-dependent manner (Fig. 3e and Supplementary Fig. 4c). Caspase-1 processing by *C. albicans* was also inhibited in cells that were treated with APDC, but not in those that received bafilomycin A (Fig. 3f and Supplementary Fig. 4d), suggesting that *C.-albicans*-dependent inflammasome triggering involves ROS production. In support of this, IL-1β secretion by *C. albicans* was impaired after NADPH oxidase inhibition with dipheylene iodoinium (Supplementary Fig. 4e)[18].

Previous studies with *Syk*$^{-/-}$ cells have demonstrated the essential role of Syk in ROS generation after fungal recognition[22]. Similarly, Syk inhibition with R406 reduced ROS production by *C. albicans* to a comparable degree to APDC treatment (Fig. 3g). In contrast, *Card9*$^{-/-}$ cells that show regular Caspase-1 activation also have normal ROS production after exposure to *C. albicans* (Supplementary Fig. 4g). Collectively, these results indicate that *C. albicans* bypasses the lysosomal cathepsin B pathway but triggers Syk-dependent ROS production and a K^+-efflux-dependent mechanism for inflammasome activation.

The molecular nature of the inflammasomes that are engaged by fungi is completely uncharacterized. Because ROS production and K^+ efflux have been linked to the activation of Nlrp3-inflammasome complexes in responses to diverse inflammatory triggers including ATP, asbestos and others[15,18,19], we proposed that *C. albicans* might engage Nlrp3. Indeed, Nlrp3-deficient cells failed to activate Caspase-1 or to produce IL-1β specifically after *C. albicans* or ATP treatment, but not after transfection with the Nlrp3-independent inflammasome activator double-stranded DNA (poly(dA:dT)[20]; Fig. 4a and Supplementary Fig. 5a, b). In line with the essential role for the adaptor Asc in coupling activated Nlrp3 or DNA recognition to Caspase-1, Asc-deficient cells also failed to process Caspase-1 or IL-1β in response to *C. albicans*, ATP or poly(dA:dT) (Fig. 4b and Supplementary Fig. 5c, d). However, in contrast to Nlrp3 and Asc, Nlrc4 (ref. 12) is dispensable for inflammasome activation by *C. albicans* (Fig. 4c). We also tested BMDCs from other Nlrp-deficient (Nlrp6 and Nlrp12; J.T., unpublished) or mutant

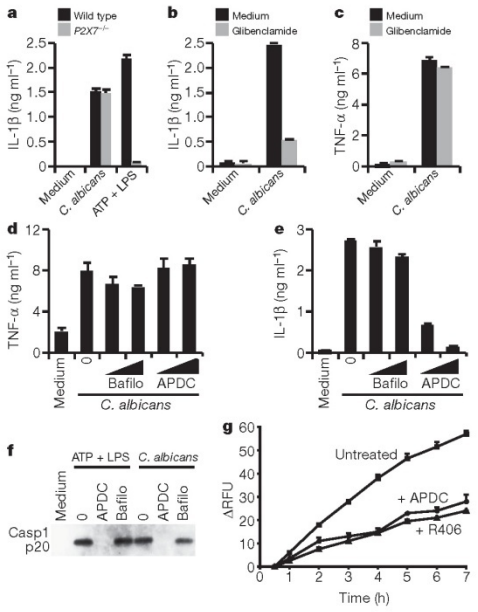

Figure 3 | Inflammasome activation by *C. albicans* involves ROS production and K^+ efflux. **a**, BMDCs from wild-type or *P2X7*$^{-/-}$ mice were stimulated with *C. albicans* or with ATP and LPS. IL-1β secretion was determined by ELISA. **b**, **c**, BMDCs were stimulated with *C. albicans* in the presence or absence of glibenclamide pretreatment. IL-1β (**b**) and TNF-α (**c**) secretion were determined by ELISA. **d**, **e**, BMDCs were left untreated (0) or treated with bafilomycin A (bafilo; 125 or 500 nM) or APDC (25 or 100 μM), and stimulated with *C. albicans*. TNF-α (**d**) and IL-1β (**e**) concentrations in the supernatant were determined by ELISA. **f**, BMDC were pretreated with bafilomycin A or APDC as in **c** and left untreated or stimulated with ATP and LPS or with *C. albicans*. Caspase-1 activation was determined by western blotting using an antibody that detects the processed p20 subunit. **g**, BMDCs were stimulated with *C. albicans* in the presence or absence of 15 min pretreatment with the ROS inhibitor APDC or the Syk inhibitor R406. ROS production was determined using the fluorescent probe 2′7′-dichlorofluorescin diacetate (H_2DCFDA). RFU, relative fluorescent units. All values in **a**–**e** and **g** are means and s.d. All results are representative of at least three independent experiments.

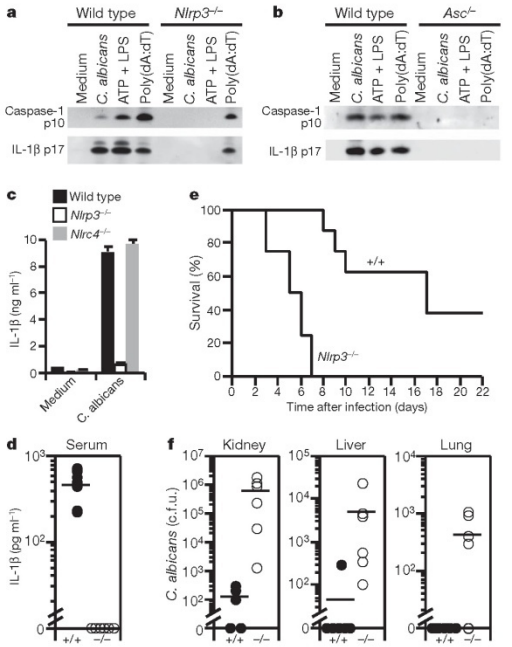

Figure 4 | The Nlrp3 inflammasome controls anti-fungal immunity. **a**, BMDCs from wild-type or *Nlrp3*$^{-/-}$ mice were stimulated with *C. albicans* or with ATP and LPS, or transfected with 2 μg ml^{-1} poly(dA:dT). Processing of Caspase-1 (p10) and production of IL-1β (p17) was determined by western blotting. **b**, BMDCs from wild-type or *Asc*$^{-/-}$ mice were treated and analysed as in **a**. **c**, BMDCs from wild-type, *Nlrp3*$^{-/-}$ or *Nlrc4*$^{-/-}$ mice were stimulated with *C. albicans*. IL-1β production was determined by ELISA. All values in **a**–**c** are means and s.d. All results are representative of at least three independent experiments. **d**–**f**, *Nlrp3*$^{-/-}$ and wild-type control mice were intravenously infected with *C. albicans* (10^5 colony forming units (c.f.u.)). **d**, IL-1β concentration in the serum of seven wild-type (+/+) and six *Nlrp3*$^{-/-}$ mice was determined after 4 h by ELISA. **e**, The frequency of viable mice is indicated over time. One out of two independent experiments with a total of 19 wild-type and nine *Nlrp3*$^{-/-}$ mice is shown. Statistical analysis was performed by log-rank test ($P < 0.0001$). **f**, Six wild-type and six *Nlrp3*$^{-/-}$ were killed 4 days after infection. *Candida albicans* titres were determined in the kidneys, livers and lungs.

mice (C57/BL6 that lack Nlrp1)[23] but found no defects in their capacity to respond to *C. albicans* (data not shown), indicating that *C. albicans* specifically engages the Nlrp3 inflammasome. To test whether Nlrp3 has a more general role in fungal responses, we also stimulated BMDCs with *Saccharomyces cerevisiae*. Similar to *C. albicans*, *S. cerevisiae* activates the inflammasome and induces IL-1β production in an Nlrp3-dependent manner (Supplementary Fig. 6a, b).

The pathophysiological role of Nlrp3 in inflammatory disorders is well established[3]. However, the *in vivo* function of Nlrp3 in host defence is largely unknown. We thus investigated the role of Nlrp3 *in vivo* by infecting mice with *C. albicans*. *Nlrp3*$^{-/-}$ mice have a severe defect in IL-1β production (Fig. 4d). Moreover, all Nlrp3-deficient animals died within 6 days after infection, whereas more than 50% of the control mice survived the challenge for over 16 days (Fig. 4e), consistent with the essential role for IL-1β in anti-fungal defence[2]. In separate experiments, we killed the animals 4 days after infection and assessed intravital fungal growth. Compared to wild-type mice, *Nlrp3*$^{-/-}$ mice had a more than 100–1,000-fold higher *C. albicans* load in the kidneys, livers and lungs (Fig. 4f), demonstrating that the Nlrp3 inflammasome mediates anti-fungal host defence *in vivo*.

In conclusion, we demonstrate that fungi can activate inflammasomes, and show, to our knowledge, the first essential role for Nlrp3 in host protection. Moreover, we describe a new mechanism of inflammasome activation that involves Syk kinase signalling. This finding may have broader implications. Syk-coupled C-type lectin receptors are emerging as important activators of inflammatory responses, and can detect exogenous or endogenous ligands[6,24,25]. In addition, prototypic pro-inflammatory crystals such as uric acid particles, which are responsible for Nlrp3-dependent gout[14], activate Syk by direct lipid membrane binding[26]. It will thus be important to study the functions of Syk in inflammasome activation in a broader context and to investigate whether the beneficial clinical effects of Syk inhibitors in inflammatory disorders[11,27] may, at least in part, be due to inhibition of the inflammasome.

METHODS SUMMARY

Mouse lines have been previously described[4,7,12,14,21,28,29]. Cells were stimulated in OptiMEM serum-free medium (Invitrogen) at 1×10^6 cells ml^{-1} in 12- or 6-well plates. Unless otherwise stated, cells were stimulated for 4 h with 5×10^6 live yeast cells per ml or with ATP (5 mM) or nigericin (3.4 μM) for 1 to 2 h. Where indicated, cells were primed with ultrapure LPS (*E. coli* K12, Invivogen, 0.5–5 ng ml^{-1}) for 3 h before addition of inflammasome activators[12]. Chemical inhibitors were added 30 min before inflammasome activation and 2.5 h after LPS priming, where applicable. Cell-free supernatants were analysed for cytokine secretion by ELISAs (BD, eBioscience or R&D) or subjected to western blot. Anti-mouse-IL-1β (R&D Systems), anti-mouse-Caspase-1 p10 (Santa Cruz, sc-514) and anti-mouse-Caspase-1 p20 (a gift from P. Vandenabeele) primary antibodies were used. For the determination of intracellular pro-IL-1β by ELISA, cells were lysed by repeated freeze–thaw cycles.

Full Methods and any associated references are available in the online version of the paper at www.nature.com/nature.

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METHODS

Mice. Mice deficient for *Caspase-1* (ref. 28), *Card9* (ref. 7), *P2X7* (ref. 4), *cathepsin B*[21], *Nlrp3* (ref. 14), *Asc*[12], *Nlrc4* (ref. 12) and *MyD88* (ref. 29) were used at 6–12 weeks of age and according to local guidelines. Syk-deficient embryonic liver chimaeras were generated as described[9].

Media and reagents. All reagents were supplied by Sigma unless otherwise stated. Cell culture reagents were from Invitrogen. FCS was from HyClone. Cytotox LDH-release assay kit was from Promega.

Cells. BMDCs, BMDMs and THP-1 cells were cultured as described[7]. Human PBMCs were isolated from whole blood of healthy, voluntary donors by standard Ficoll-Hypaque density gradient centrifugation (Biochrom).

Cell stimulation. Cells were stimulated in OptiMEM serum-free medium at 1×10^6 cells ml^{-1} in 12- or 6-well plates. *Candida albicans* cells were maintained on selective Chromagar plates (BD Biosciences). Before stimulation, they were expanded overnight on Columbia agar plates (BD Bioscience) at 30 °C. Viable *C. albicans* cells were rinsed off the plate, washed twice with PBS, left unopsonised and used directly for stimulation as described[7]. *Saccharamocyes cerevisiae* were prepared similarly. *Candida albicans* hyphae were prepared by incubating the fungal cells overnight in RPMI at 37 °C. Fungal yeast or hyphae were killed by incubation in a boiling water bath for 30 min. Unless otherwise stated, cells were stimulated for 4 h with 5×10^6 yeast cells per ml or equivalent amounts of hyphae. Cells were stimulated with ATP (5 mM) or nigericin (3.4 μM) for 1 to 2 h, unless otherwise stated. *Salmonella typhimurium* (m.o.i. 10)[12] and double-stranded DNA (poly(dA-dT)•poly(dA-dT), known as poly(dA:dT), 2 μg ml^{-1})[20] were used as described. Where indicated, cells were primed with ultrapure LPS (*E. coli* K12, Invivogen, 0.5–5 ng ml^{-1}) for 3 h before the addition of inflammasome activators as described[12]. Cell supernatants were analysed for cytokine secretion by ELISA (BD, eBioscience R&D Systems or MBL) or subjected to western blot analysis. For the determination of intracellular pro-IL-1β by ELISA, cells were lysed by repeated freeze–thaw cycles.

Chemical inhibitors. Chemical inhibitors were added 30 min before cell stimulation (2.5 h after LPS priming, where applicable). Pan-caspase-inhibitor (z-VAD-fmk, Calbiochem) was used at 25 ng ml^{-1}. R406, provided as a gift from Rigel, Inc. was used at 1 μM or as indicated. The ROS inhibitor APDC (Alexis) was used at 50 μM or as indicated. Bafilomycin A was used at 100 nM or as indicated. Dipheylene iodoinium was used at 10 μM, glibenclamide at 25 μM. As a control for specificity and toxicity, we confirmed that the inhibitors did not interfere with LPS- or *C.-albicans*-dependent TNF-α and/or pro-IL-1β production.

ROS assay. The ROS indicator H_2DCFDA (20 μM, Fluka) was added to the cells in HBSS 30 min before stimulation according to manufacturer's instructions. Fluorescence was recorded in 96-well plates over time with a Titertek FluoroskanII using a FITC filter (excitation 485 nm, emission 538 nm).

Western blotting. Cell supernatants or cell extracts were subjected to standard western blot techniques as described[30]. Proteins from cell-free supernatants were extracted by methanol/chloroform precipitation. Anti-mouse-IL-1β (R&D Systems), anti-mouse-Caspase-1 p10 (Santa Cruz, sc-514) and anti-mouse-Caspase-1 p20 (a gift from P. Vandenabeele) primary antibodies were used.

Abkürzungsverzeichnis

3pRNA	dsRNA, die am 5'-Ende ein Triphosphat trägt
AIM2	Absent in Melanoma; intrazellulärer DNA-Rezeptor
APC	Antigen präsentierende Zelle
Asc	Apoptosis-associated speck-like protein containing a CARD
ATP	Adenosintriphosphat
Bcl10	B-cell lymphoma/leukemia 10
BCR	B cell Rezeptor
Card9	Caspase recruitment domain-containing protein 9
CARD	Caspase recruitment domain
CD	Cluster of differentiation
cDC	konventionelle DC
CLEC	C-type-Lektine
CpG	Cytosine-Phosphat-Guanine, DNA-Motif
DAMP	danger-associated molecular pattern
DNA	Deoxyribonucleic acid
dsRNA	doppelsträngige RNA
IFN	Interferon
IL	Interleukin
IPAF	ICE-Protease activating factor
IRF	interferon regulatory transcription factor
LGP2	laboratory of genetics and physiology 2
LRR	Leucin rich repeat
Malt-1	Mucosa-associated lymphoid tissue lymphoma translocation protein 1
MAP	Mitogen-activated protein
MAPK	MAP Kinase
MAVS	mitochondrial antiviral signaling protein
MBL	Mannose-bindendes Lektin
MDA-5	Melanoma differentiation-associated protein 5.
MHC	Major-histocompatibility-complex
MyD88	Myeloid differentiation primary response gene (88)
NACHT	NAIP, CIITA, HET-E and TP1-Domäne
NFκB	nuclear factor kappa-light-chain-enhancer of activated B cells
NK-Zelle	natürliche Killerzelle
NLR	NOD-like-Rezeptoren; auch NOD domain, LRR protein
Nlrp3	NLR protein 3
NOD	nucleotide-binding oligomerization
PAMP	Pathogen-associated molecular pattern
pDC	plasmazytoide DC
PRR	Pattern recognition receptor
PYD	pyrin domain
RIG-I	retinoic acid-inducible gene 1 protein
RLH	RIG-like-Helikasen
RNA	Ribonucleic acid
ROS	reactive oxygen species
ssRNA	einzelsträngige (single stranded) RNA
Syk	Spleen tyrosine kinase
TCR	T-Zell Rezeptor
TGF-β	Transforming growth factor beta
Th	T helper
TLR	Toll-like-Rezeptor
TRIF	TIR-domain-containing adapter-inducing interferon-β

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