Mass spectrometry

Experiments were performed using a standard protein identification strategy using mass spectrometry [33]. Briefly, gels were cut into pieces, followed by reduction using dithiothreitol (DTT), and alkylation using iodoacetamide (IAA). Bands were digested with trypsin overnight and extracted peptides were subsequently speed vacumed to reduce volume and remove organic solvent. Peptides were chromatographically resolved on-line using a Zorbax 300SB-C18 column and 1200 series high performance liquid chromatography (HPLC, Agilent Technologies) and analyzed using a 6340 LCMS ion trap mass spectrometer (Agilent Technologies, Palo Alto, CA) in the National Jewish Health Proteomics Facility. The mass spectrometry system includes a HPLC-chip interface (Agilent Technologies). Raw data was extracted and searched using the Spectrum Mill search engine (Rev A.03.03.038 SR1, Agilent Technologies, Palo Alto, CA). "Peak picking" was performed within SpectrumMill with the following parameters: signal-to-noise set at 25:1, a maximum charge state of 4 allowed ($z \leq 4$), and the program is directed to attempt to "find" a precursor charge state. During searching the following parameters were applied: IPI human database (IPI Human rel. 3.28, 16-APR-2007) carbamidomethylation as a fixed modification, trypsin, maximum of 1 missed cleavage, precursor mass tolerance +/- 1.7, product mass tolerance +/- 0.7, and maximum ambiguous precursor charge = 3. Data was evaluated and protein identifications were considered significant if the following confidence thresholds were met: minimum of 2 peptides per protein, protein score > 20, individual peptide scores of at least 10, and Scored Percent Intensity (SPI) of at least 70%. The SPI provides an indication of the percent of the total ion intensity that matches the peptide's MS/MS spectrum. A reverse (random) database search was simultaneously performed and manual inspection of spectra was used to validate the match of the spectrum to the predicted peptide fragmentation pattern, hence increasing confidence in the identification. Standards are run at the beginning of each day and at the end of a set of analyses for quality control purposes.

Present in Alum Only - Fibrinogen chains	Number of Unique Peptides Identified		
	Nodules, 2 hours	Nodules, 18 hours	Normal serum
Fibrinogen alpha	5	5	Undet.
Fibrinogen beta	8	11	Undet.
Fibrinogen gamma	6	7	Undet.

Present in Alum Only - Histones	Number of Unique Peptides Identified		
	Nodules, 2 hours	Nodules, 18 hours	Normal serum
Histone H1	4	3	Undet.
Histone H2A	3	2	Undet.
Histone H2B	2	Undet.	Undet.
Histone H3	2	1	Undet.
Histone H4	4	4	Undet.

Present in Alum Only - Primarily at 2 hours	Number of Unique Peptides Identified		
	Nodules, 2 hours	Nodules, 18 hours	Normal serum
Apolipoprotein H	2	Undet.	Undet.
Basigin	3	Undet.	Undet.
Calpactin I (Annexin)	2	Undet.	Undet.
Malate dehydrogenase 2	2	Undet.	Undet.
Mast cell carboxypeptidase A	2	Undet.	Undet.

Present in Alum Only - Primarily at 18 hours	Number of Unique Peptides Identified		
	Nodules, 2 hours	Nodules, 18 hours	Normal serum
Annexin A1 (Lipocortin I)	Undet.	3	Undet.
Antithrombin-III	Undet.	3	Undet.
Apolipoprotein E	1	4	Undet.
ATP synthase alpha	2	2	Undet.
ATP Synthase beta	1	2	Undet.
Calgranulin B	1	2	Undet.
Myeloid bactenecin	Undet.	2	Undet.
Myeloperoxidase	Undet.	2	Undet.
Semen coagulation protein	2	8	Undet.
Spi A3N	2	5	Undet.
Vitronectin	Undet.	3	Undet.

Present in Alum and Normal Serum	Number of Unique Peptides Identified		
	Nodules, 2 hours	Nodules, 18 hours	Normal serum
Actin	5	5	5
Albumin	11	10	28
Alpha-1-antitrypsin	5	Undet.	16
Alpha-2-HS gp (fetuin)	3	3	2
Annexin A2 (Lipocortin II)	1	3	1
Apolipoprotein A-I	3	2	8
Clusterin/Apolipoprotein J	2	8	1
Hemoglobin beta	5	5	7
Hemopexin	2	Undet.	8
Ig heavy chain (Igh-6)	7	Undet.	2
Transthyretin	3	Undet.	4

Table S1. Nodule proteins identified by mass spectrometry

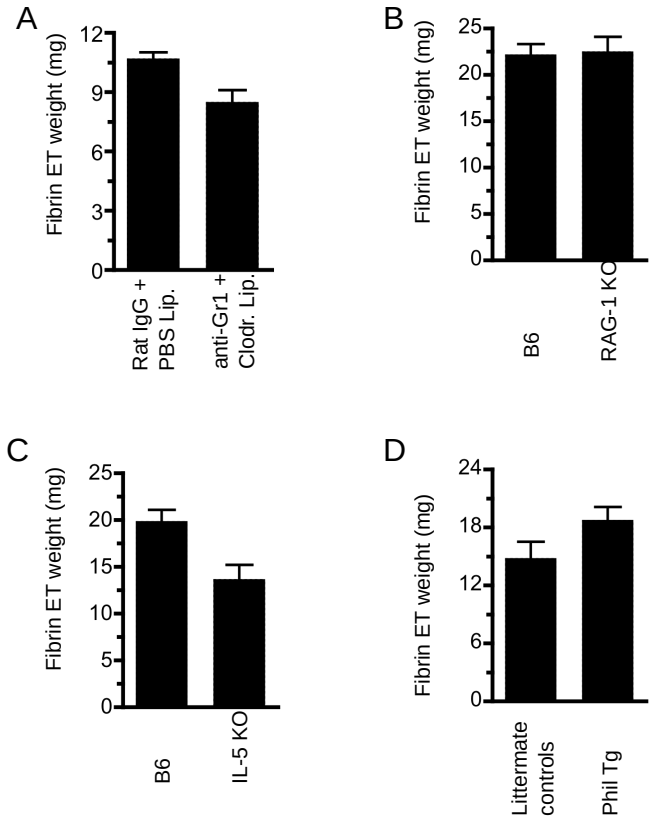

Figure S1. Macrophages, T cells, B cells and eosinophils are dispensable for fibrin ET formation

A. C57BL/6 mice were depleted of neutrophils and macrophages by injection of 200 ug of anti-Gr1 i.v. and 50 ul of clodronate-containing liposomes i.p., or control antibody and liposomes. One day later, mice were injected with 4 mg of Alhydrogel and evaluated for fibrin ET formation 5 hours later.

B. RAG-1 KO mice were injected with Alhydrogel and evaluated for fibrin ET formation 5 hours later.

C. IL-5 KO mice were injected with Alhydrogel and evaluated for fibrin ET formation 5 hours later. Note that fibrin ET formation was decreased by 24%, which was not statistically significant (p=0.23).

D. Phil Tg mice, which express diphtheria toxin under the control of the eosinophil peroxidase (EPO) promoter, were injected with Alhydrogel and evaluated for fibrin ET formation 5 hours later.

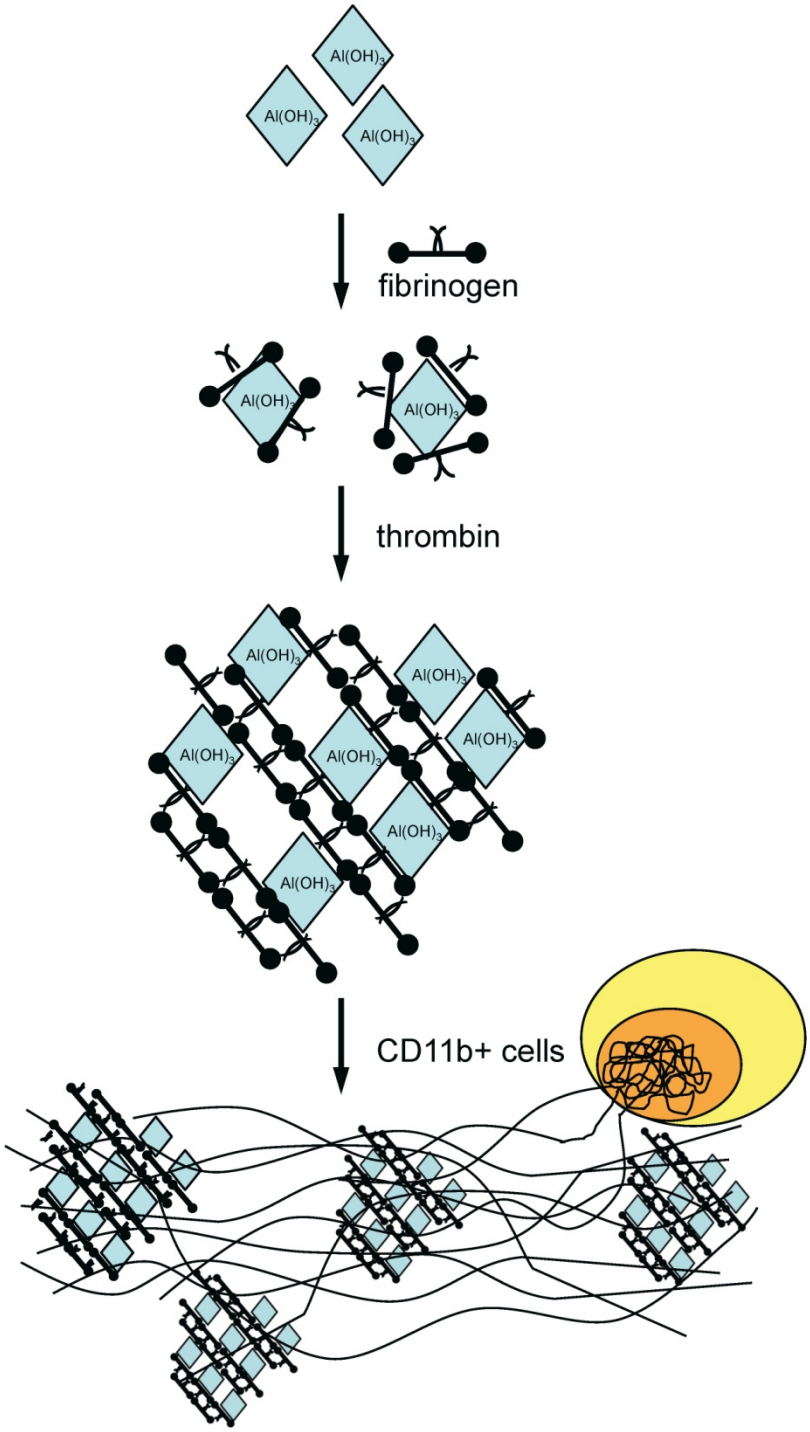

Figure S2. Model of aluminum adjuvant fibrin ET formation
Step 1: Upon exposure to extracellular fluid, aluminum adjuvant becomes coated by fibrinogen.
Step 2: Thrombin cleaves adsorbed fibrinogen into fibrin, causing particles to aggregate.
Step 3: $CD11b^{+}$ cells extrude chromatin, coating and cross-linking the aggregates to form into fibrin-dependent extracellular traps (fibrin ETs).