PNAS RESEARCH ARTICLE | IMMUNOLOGY AND INFLAMMATION OPEN ACCESS

Tattoo ink induces inflammation in the draining lymph node and alters the immune response to vaccination

Arianna Capucetti[a,1], Juliana Falivene[a,b,1], Chiara Pizzichetti[a,c], Irene Latino[a], Luca Mazzucchelli[d], Vivien Schacht[e], Urs Hauri[f], Andrea Raimondi[g], Tommaso Virgilio[a], Alain Pulfer[a], Simone Mosole[h], Llorenç Grau-Roma[i], Wolfgang Bäumler[j], Martin Palus[k,l], Louis Renner[a], Daniel Ruzek[k,l,m], Gabrielle Goldman Levy[n], Milena Foerster[o], Kamil Chahine[a,c], and Santiago F. Gonzalez[a,2]

Affiliations are included on p. 10.

Edited by Alberto Mantovani, Humanitas University, Pieve Emanuele, Italy; received April 28, 2025; accepted September 12, 2025

Despite safety concerns regarding the toxicity of tattoo ink, no studies have reported the consequences of tattooing on the immune response. In this work, we have characterized the transport and accumulation of different tattoo inks in the lymphatic system using a murine model. Upon quick lymphatic drainage, we observed that macrophages mainly capture the ink in the lymph node (LN). An initial inflammatory reaction at local and systemic levels follows ink capture. Notably, the inflammatory process is maintained over time, as we observed clear signs of inflammation in the draining LN 2 mo following tattooing. In addition, the capture of ink by macrophages was associated with the induction of apoptosis in both human and murine models. Furthermore, the ink accumulated in the LN altered the immune response against two different types of vaccines. On the one hand, we observed a reduced antibody response following vaccination with an messenger ribonucleic acid (mRNA)-based severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) vaccine, which was associated with a decreased expression of the spike protein in macrophages in the draining LN. In contrast, we observed an enhanced response when vaccinated with influenza vaccine inactivated by ultraviolet (UV) radiation. Considering the unstoppable trend of tattooing in the population, our results are crucial in informing the toxicology programs, policymakers, and the general public regarding the potential risk of the tattooing practice associated with an altered immune response.

immunotoxicology | covid-vaccination | inflammation

Significance

In this study, we characterized the immune responses to the tattoo ink accumulating in the lymph nodes (LNs). This is very relevant as tattoo ink commonly reaches and persists in this organ in most tattooed subjects, often lifelong. We have observed that ink is retained within phagocytic cells, which undergo cell death and induce a prominent and long-term inflammatory response, with elevated levels of proinflammatory cytokines in LNs up to 2 mo after tattooing. Furthermore, we observed that tattoo ink at the vaccine injection site modulated immune responses in a vaccine-specific manner, with a reduced response to the COVID-19 vaccine and an enhanced response to the UV-inactivated influenza vaccine, reflecting differences in the mechanisms of action between these vaccine classes.

Tattooing has become widespread, particularly for young people. According to the latest studies, it is estimated that globally, almost one out of five individuals have tattoos (1), with the United States having the highest prevalence with more than 30% of tattooed individuals (2, 3). During the tattooing process, repeated penetration by needles into the dermal layer of the skin introduces different types of pigments following a pattern. The permanence of tattoos is achieved by using pigments that are not readily soluble in bodily fluids, often formed by a complex mixture of pigment binders, solvents, and additives (4). While carbon black is the primary pigment used in black tattoos, colored tattoos typically contain industrial organic pigments originally intended for plastics, varnishes, or paints (5, 6).

Despite the increasing prevalence of tattooing, the regulation of tattoo inks is less stringent than that of the pharmaceutical industry or other products intended for human use. Even though toxicological data may be available for some ink ingredients (7), studies on in vivo interactions of the ink components and their fate within the body are rare. The lack of comprehensive regulations has prompted the identification of risks associated with tattooing and the development of specific information to ensure that individuals can make informed decisions before undergoing this procedure (8, 9). In Europe, the composition of the ink has been regulated since 2022 by the "Registration, Evaluation, Authorization, and Restriction of Chemicals" (REACH) program, which intends to harmonize the legislation through the member states (8, 9). However, the potential health implications of this form of body art have become a subject of increasing concern (5).

Previous studies have shown that macrophages are the primary cells responsible for ink uptake in the skin (10). In conjunction with tattooed skin, pigmented and enlarged lymph nodes (LN) and several adverse immune-related reactions have been reported in tattooed individuals for decades (11–17). Notably, studies performed in mouse models have shown the accumulation of tattoo ink in the LN draining the tattoo site, mimicking what has been observed in humans and primates (18, 19). Despite the central role of the LN in the development of appropriate immune responses, the mechanisms and potential consequences behind the immunological reactions upon tattooing remain unknown. Moreover, many

The authors declare no competing interest.

Where authors are identified as personnel of the International Agency for Research on Cancer/or the World Health Organization, the authors alone are responsible for the views expressed in this article and the views do not necessarily represent the decisions, policy, or views of the International Agency for Research on Cancer/or the World Health Organization.

This article is a PNAS Direct Submission.

[1]A.C. and J.F. contributed equally to this work.

[2]To whom correspondence may be addressed. Email: santiago.gonzalez@irb.usi.ch.

This article contains supporting information online at https://www.pnas.org/lookup/suppl/doi:10.1073/pnas.2510392122/-/DCSupplemental.

Published November 25, 2025.

concerns regarding complications from vaccination and other medical procedures in tattooed individuals have been raised over the years, especially during the mass vaccination against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). Although it is recommended to avoid vaccination on fresh tattoos, no specific studies have evaluated the impact of tattoos on vaccination efficacy or the risk of infections associated with tattoos (20).

In summary, this study elucidates the transport and accumulation of tattoo inks in the draining LN, characterizing the inflammatory response to tattooing and evaluating how tattooing affects the humoral immune response to different classes of vaccines, an mRNA-based COVID-19 vaccine and a UV-inactivated influenza vaccine. Our work provides a comprehensive analysis of the immunotoxicology of tattooing.

Results

The Lymphatic System Transports Tattoo Ink, Which Accumulates in the Synodical Areas of the Draining LN. We selected three of the most used colors of ink, black, red, and green, from one of the major world providers (Intenze) and confirmed that the composition of the inks was concordant with the REACH regulation (21) (*SI Appendix*, Table S1). Then, we set up a mouse model, tattooing an area of around 25 mm^2 in the footpad of the mice (Fig. 1 *A* and *E*, *Upper*). Next, we confirmed by electron microscopy (EM) the presence of ink in both the epidermis and the dermis of the skin, associated with cells resembling keratinocytes, macrophages, and the collagen matrix (*SI Appendix*, Fig. S1*A*). To evaluate the transport of unretained ink from the skin area to the draining LN, we surgically exposed the mouse hindlimb (22) and we performed intravital imaging following footpad tattooing (Fig. 1*B*). We observed that the ink disseminates exclusively via popliteal lymphatic vessels, reaching an initial peak at 10 min following tattooing (Fig. 1 *C* and *D*). Furthermore, the transport of ink via the lymphatic system was associated with a prominent accumulation in the popliteal LN (pLN) observed at 24 h posttattooing (p.t.) for all the tested inks (Fig. 1 *E*, *Middle*). Interestingly, we also observed the presence of ink in the lumbar LN with two of the tested inks (black and red), suggesting that unretained ink disseminates further via the efferent lymphatics (Fig. 1 *E*, *Lower*). Next, we performed confocal microscopy to determine the pLN areas where the ink pigments accumulate (Fig. 1*F*). In addition, we measured the mean fluorescent intensity (MFI) associated with the ink, observing that at 24 h p.t., there was a significant increase in the size of the pLN and in the area occupied by the ink, compared to the PBS-tattooed control (Fig. 1*G*). Moreover, at this point, the pigment signal mainly accumulated in the subcapsular and medullary areas of the LN (Fig. 1*H*). To evaluate the dynamics of ink pigment accumulation over extended periods, we examined the presence of pigments at 2 mo p.t., detecting an increase in the signal in both the popliteal and lumbar LNs (Fig. 1 *I*–*K* and *SI Appendix*, Fig. S1*B*). Furthermore, we observed that ink was also present in the paracortical area of the LN (*SI Appendix*, Fig. S1*B*). Remarkably, we observed a similar distribution in all the human samples from LN biopsies evaluated collected from deidentified patients who had a tattoo in the LN-drained area at least several months before the biopsy (*SI Appendix*, Fig. S1*D*).

The Capture of Ink in the LN Is Mainly Associated with Medullary Macrophages (MM). To evaluate the cell type responsible for the capture of the ink particles in the sinusoidal areas of the pLN we performed confocal staining of the main phagocytic populations: subcapsular sinus macrophages (SSM; CD169+, CD11cdim, and F4/80–), MM (CD169+, CD11c–, and F4/80+), and dendritic cells (DC; CD169–, CD11c+, and F4/80–) at 24 h and 2 mo p.t. with black (Fig. 2 *A* and *B*), red (Fig. 2 *C* and *D*), and green ink (Fig. 2 *E* and *F*). Quantification of the images demonstrated that MM are the primary cell type responsible for the ink particle uptake, compared to the other two cell types analyzed at an initial stage and at 2 mo p.t. (Fig. 2 *B*, *D*, and *F*). Furthermore, we confirmed the identity of these cells by using a transgenic mouse model expressing the CX3CR1 (*SI Appendix*, Fig S2*A*). Additionally, we observed that DC can capture some ink. However, most ink particles were associated with macrophages (Fig. 2 *B*, *D*, and *F* and *SI Appendix*, Fig. S2*B*). Next, we performed resin embedding of the pLN (Fig. 2 *G* and *H*) and analyzed the samples using transmission EM. We confirmed that most ink particles were associated with phagocytic cells displaying a macrophage-like morphology at 24 h p.t. (Fig. 2 *H* and *I*). Moreover, we could observe multiple phagocytic vacuoles containing electrodense material (Fig. 2*I*). When we evaluated the structure of the same cells at 2 mo p.t., we observed an increase in the size of the cells and the amount of ink associated with macrophages in the medullary region and the formation of giant cells (GCs) with multiple phagocytic vacuoles containing ink particles (Fig. 2 *K* and *L*). Interestingly, we confirmed that tattoo ink particles are also associated with CD68+ or CD163+ macrophages in two biopsies of LNs from tattooed patients (*SI Appendix*, Fig. S2 *C* and *F*, respectively) and observed numerous GCs containing ink particles deposited, showing a similar morphology to the ones described in the murine model (*SI Appendix*, Fig. S2*E*)

Tattoo Ink Induces the Death of Macrophages. To study the immune cell dynamics in the draining LN following the arrival of tattoo ink, we measured the total number of cells in the pLN of mice by Fluorescence-activated cell sorting (FACS) during the first 5 d following tattooing. We observed a significant increase in the total number of macrophages during the first six h p.t., with the red and black ink, which were followed by a significant decrease at 12 h and 24 h p.t., respectively (Fig. 3*A* and *SI Appendix*, Fig. S3 *A*, *Right*). Regarding the green ink, macrophage decrease was only observed at 120 h p.t. (*SI Appendix*, Fig. S3 *A*, *Left*). Thus, we hypothesized that the observed disappearance might be associated with the toxicity of the unretained ink particles in the pLN. Furthermore, we observed by EM that some of the ink-associated macrophages in the medullary sinuses of the pLN undergo morphological changes such as the loss of plasma membrane integrity (Fig. 3 *B*, *Left*) or the formation of blebs (Fig. 3 *B*, *Right*), characteristic of cell death stages (23). Therefore, to better characterize the mechanism of ink-associated cell death, we isolated mesenchymal stem cells from the bone marrow of mice, differentiated them into macrophages, and added different concentrations of the three inks (*SI Appendix*, Fig. S3*B*). First, we confirm a concentration-dependent uptake of all the inks by in vitro-cultured macrophages that resembles the one observed in the in vivo studies (Fig. 3*C* and *SI Appendix*, Fig. S3*C*). Then, to confirm the presence of ink-induced cell death we cultured the ink-treated macrophages in the presence of propidium iodide (PI), a marker of necrosis, and quantified the number of PI^+ cells using imageXpress® (Fig. 3 *D* and *E*). Following this approach, we observed a significant increase in the number of PI^+ cells at 12 h post–ink addition (p.i.a) with the red and the black inks compared to the control group. However, we could not see any toxic effect in the samples treated with green ink (Fig. 3*E*). Moreover, we confirmed by flow cytometric analysis that all the tested inks induced different levels of apoptosis (Annexin V^+, PI^- cells) in the macrophages during the first 48 h p.i.a. Indeed, the addition

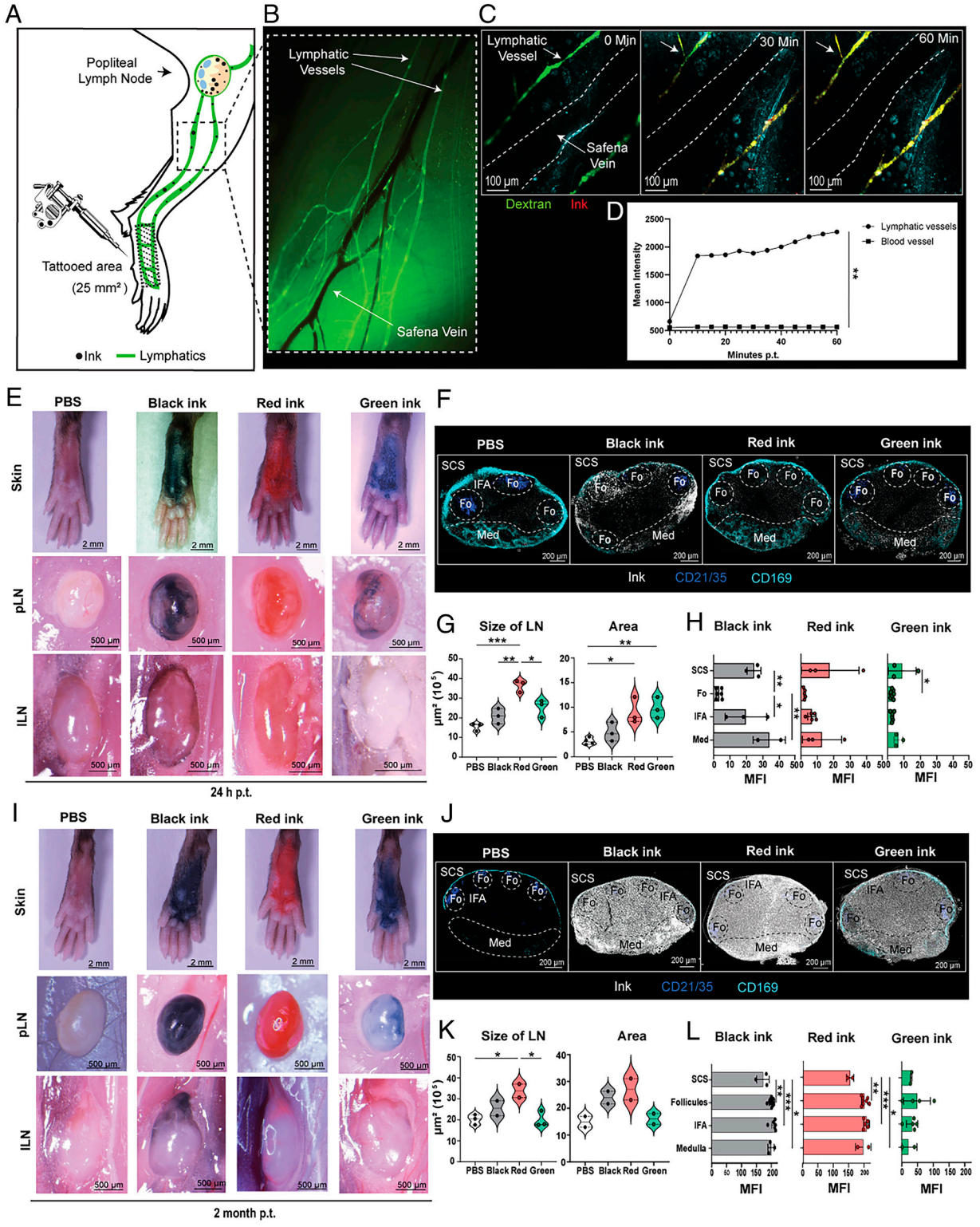

Fig. 1. Tattoo ink is transported by the lymphatic system and accumulates in the draining lymph node. (*A*) Graphic representation of the lymphatic drainage in the tattoo murine model employed. (*B*) Fluorescence micrograph showing the imaged area located in the calf of the mouse. (*C*) Representative intravital 2-photon micrographs acquired at 0-, 30-, and 60-min posttattooing with red ink. (*D*) Quantification of the mean intensity of the red ink autofluorescence inside the lymphatic and the blood vessels over 60 min following tattooing. Pearson correlation analysis [r = 0.7690, *P* (two-tailed) = 0.0035]. (*E*) Snapshots showing the tattooed area on the mouse footpad and the ink accumulation in the draining LNs at 24 h after tattooing with commercial True Black, Bright Red, or Pure Green inks. (*F*) Representative confocal micrographs following the ink distribution in the different areas of the LN at 24 h p.t.: SCS stands for subcapsular sinus; Fo, B cell follicle; Med, medullary region; IFA, interfollicular area. (*G*) Violin plot representing the average size of the pLN and the area occupied by ink signal at 24 h p.t. (*H*) Quantification of the MFI of the inks in the different regions of the LN at 24 h p.t. (*I*) Snapshots showing the tattooed area on the mouse footpad and the ink accumulation in the draining LNs at 2 mo after tattooing with commercial True Black, Bright Red, or Pure Green inks. (*J*) Representative confocal micrographs following the ink distribution in the different areas of the LN at 2 mo p.t: SCS stands for subcapsular sinus; Fo, B cell follicle; Med, medullary region; IFA, interfollicular area. (*K*) Violin plot representing the average size of the pLN and the area occupied by ink signal at 2 mo p.t. (*L*) Quantification of the MFI of the inks in the different regions of the LN at 2 mo p.t. In (*G* and *H* and *K* and *L*), one out of two or more independent experiments is shown. Popliteal lymph node (pLN); iliac lymph node (ILN). Data are presented as mean ± SD or Median and 25th (*Bottom*) and 75th (*Top*) percentiles. One-way ANOVA followed by Bonferroni correction for multiple comparisons. Differences between groups were considered significant with *P*-value < 0.05 (*$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$).

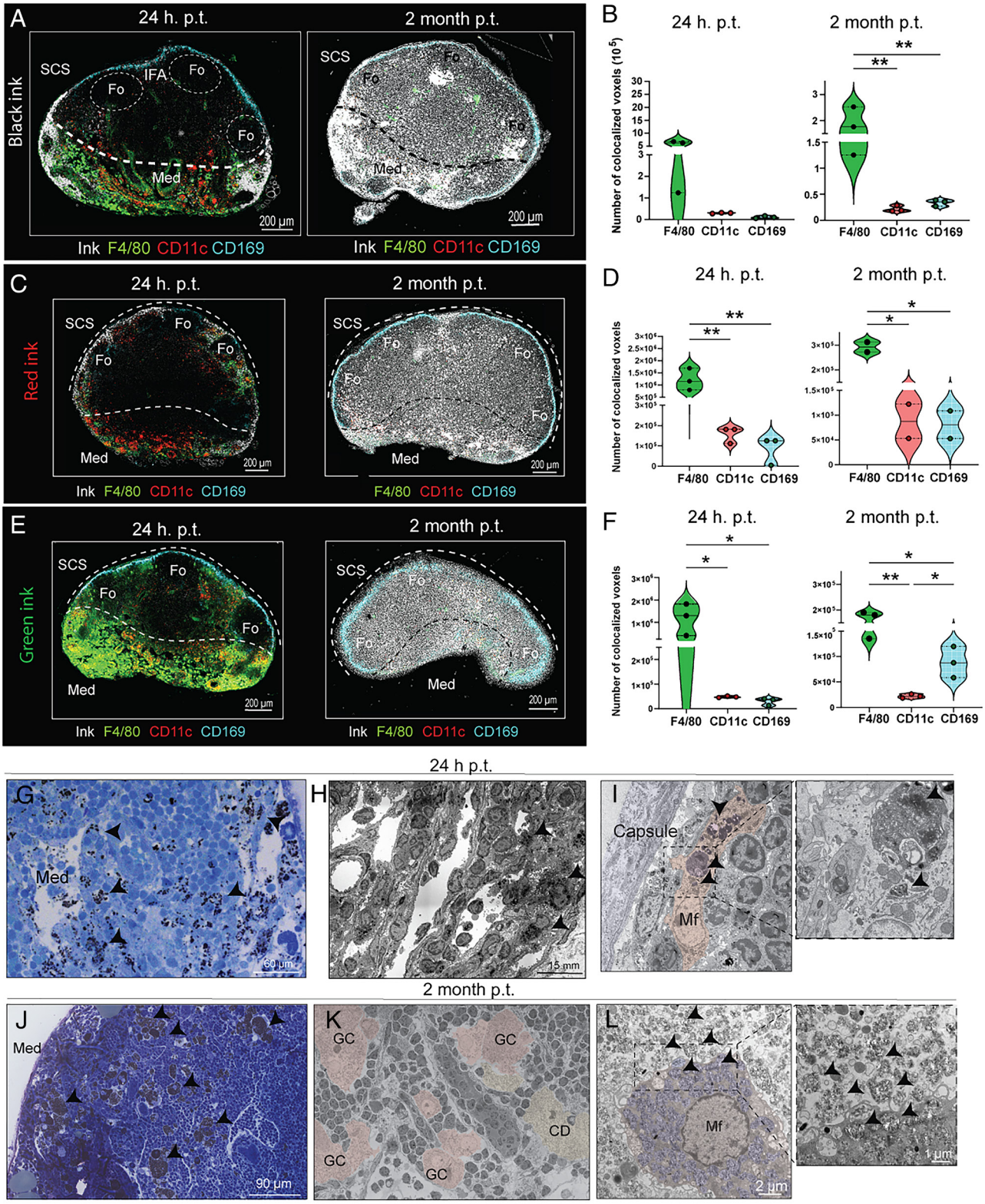

Fig. 2. MM capture and retain tattoo ink. (*A*) Representative confocal micrographs showing the colocalization of black ink with the main phagocytic populations in the LN at 24 h (*Left*) and 2 mo (*Right*) p.t. (*B*) Violin plot representing the quantification of colocalized voxels of black ink with the major phagocytic populations in the draining LN at 24 h (*Left*) and 2 mo (*Right*) p.t. *(C)* Representative confocal micrographs showing the colocalization of red ink with the main phagocytic populations in the LN at 24 h (*Left*) and 2 mo (*Right*) p.t. *(D)* Violin plot representing the quantification of colocalized voxels of red ink with the major phagocytic populations in the draining LN at 24 h (*Left*) and 2 mo (*Right*) p.t. *(E)* Representative confocal micrographs showing the colocalization of green ink with the main phagocytic populations in the LN at 24 h (*Left*) and 2 mo (*Right*) p.t. *(F)* Violin plot representing the quantification of colocalized voxels of green ink with the major phagocytic populations in the draining LN at 24 h (*Left*) and 2 mo (*Right*) p.t. (*G*) Toluidine Blue staining of the pLN at 24 h and 2 mo p.t. (*J*). Black arrowheads indicate the presence of ink. (*H*) Representative EM micrographs of the medullary sinuses at 24 h and 2 mo p.t. (*K*), showing the presence of macrophages with multiple ink-containing vacuoles at 24 h (*I*) and 2 mo p.t. (*L*). Giant cell (GC); Cell debris (CD); Medulla (Med); Macrophage (Mf). One out of two or more independent experiments is shown. Data are presented as median and 25th (*Bottom*) and 75th (*Top*) percentiles. One-way ANOVA followed by Bonferroni correction for multiple comparisons (*$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$).

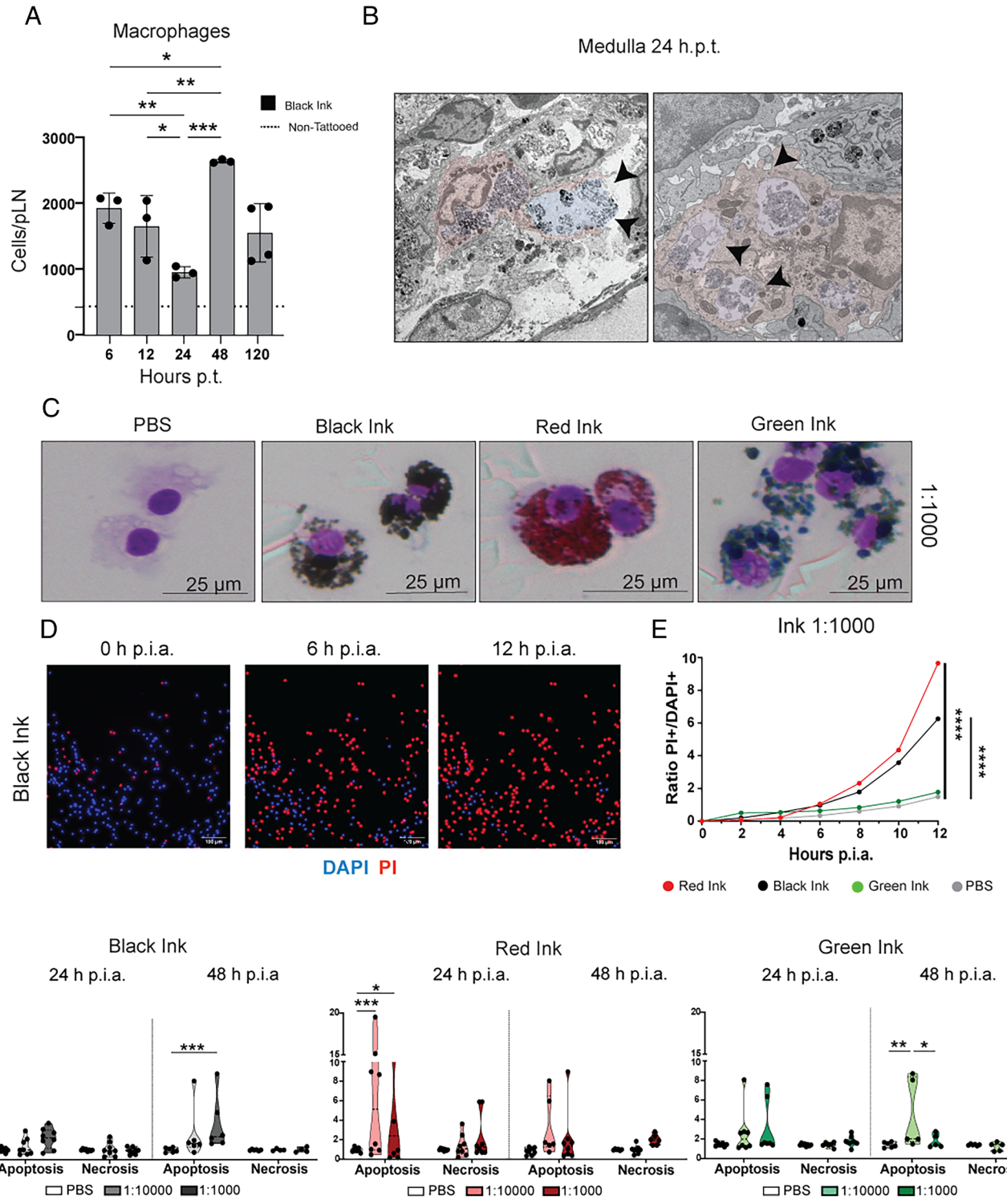

Fig. 3. Tattoo ink induces macrophage death. (*A*) Absolute number of macrophages in the pLN of tattooed animals during the 120 h following footpad tattooing with black ink. One out of two independent experiments is shown. (*B*) Representative EM micrographs of ink-captured macrophage-like cells (fake colored in red). Black arrowheads show signs of distress, such as the loss of plasma membrane integrity and the formation of blebs. (*C*) Representative cytospin images showing the capture of black, red, and green ink (ink dilution 1:1,000) by bone marrow–derived macrophages. (*D*) Representative snapshots of DAPI and PI stained macrophages at 6 and 12 h p.i.a. and quantification plot (*E*) showing the ratio of PI^+ on $DAPI^+$ cells after adding inks. Pearson correlation analysis of black vs. PBS groups [r = 0.9933, *P* (two-tailed) < 0.0001], red vs. PBS groups [r = 0.9817, *P* (two-tailed) < 0.0001], and green vs. PBS groups [r = 0.9739, *P* (two-tailed) = 0.0002]. (*F*) Flow cytometric analysis showing the ratio of apoptotic (Annexin V^+, PI^-) and necrotic (Annexin V^+, PI^+) cells at 24 h and 48 h postaddition of black, red, and green ink compared to the PBS controlled group. Data are presented as mean ± SD. One-way (*A*) or Two-way (*F*) ANOVA followed by Bonferroni correction for multiple comparisons (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, and ****$P < 0.0001$).

of red ink was followed by a significant sevenfold induction of apoptosis at 24 h, while black and green inks induced significant levels of apoptosis at 48 h p.i.a. (Fig. 3*F*). Importantly, we observed that macrophages differentiated from human peripheral blood mononuclear cells (PBMC) (*SI Appendix*, Fig. S3*D*), showed similar trends, in terms of ink capture and ink-induced cell death, when cultured with all the selected inks (*SI Appendix*, Fig. S3 *E*–*H*). However, some differences were observed regarding the time of

death induction. Both black and green inks induced significant levels of apoptosis already at 24 h p.i.a. (*SI Appendix*, Fig. S3 *F* and *H*, respectively), while the red ink induced necrosis at 24 h, followed by apoptosis at 48 h p.i.a (*SI Appendix*, Fig. S3*G*).

Tattoo Ink Induces an Acute and Long-Lasting Inflammation in the Draining LN. Considering the relationship between the induction of cell death pathways and inflammation (24), we hypothesized that ink-induced macrophage cell death could be associated with inflammation in the draining LN. Therefore, to evaluate the swelling of the LN, we estimated the total number of cells after tattooing with black ink, observing a significant increase at 48 h p.t., that followed a continuous growth for at least the first 240 h p.t (Fig. 4*A*). We observed similar dynamics in the number of $CD45^+$ cells recruited to the pLN when the tattoo was made with the red and the green inks (*SI Appendix*, Fig. S4*A*). The significant increase in the number of cells at 48 h p.t. was further confirmed by FACS analysis for B, T, Natural Killer (NK) cells, and conventional DCs, following tattooing with black ink (Fig. 4*B*), as well as with red and green inks (*SI Appendix*, Fig. S4 *B* and *C*, respectively). To further characterize the inflammatory response, we collected the lymph from the pLN during the first 240 h following tattooing with black ink, and we measured the levels of inflammatory cytokines and chemokines at different instances. According to the trend of the expression observed, we could identify an acute phase, characterized by the upregulation of the cytokines IL-6, CXCL1, CCL2, and CCL3, which peak at 6 to 12 h p.t., and return to basal levels after the first 240 h p.t. (Fig. 4 *C*, *Upper* graphs).

Furthermore, we identified a second group of molecules, including eotaxin, CXCL13, IL-1α, and CXCL9, characterized by their long-term expression, which remains significantly elevated during the first 240 h p.t. (Fig. 4 *C*, *Lower* graphs). Additionally, we observed similar expression patterns when tattooing with both red and green inks (*SI Appendix*, Fig. S4*D*). To determine whether the observed inflammation also occurs systemically, we measured the expression of inflammatory mediators in blood at early (first 5 d) and late (2 mo) times posttattooing. Interestingly, we found that the early peak of inflammation previously described was also detected systemically, with elevated levels of the inflammatory cytokines IL-6, TNF-α and IL-1β, and the chemokines CCL2, CXCL9, and CXCL13, measured in the blood during the first 24 h following tattooing with all three tested inks (Fig. 4*D*). The levels of most of these molecules were only transiently elevated. However, the levels of the alarmin IL-1α remain elevated in the blood from all tattooed groups 2 mo after tattooing (Fig. 4*D*). Further, to characterize the long-term inflammatory response in the draining LN, we measured the numbers of B, T, NK cells, and DCs at 2 mo p.t. with black ink Fig. 4*D*, observing a significant increase in all the cell types at this time point, compared with the control groups (Fig. 4*E*). We could also observe a tendency to have elevated levels of these cells in animals tattooed with red and green inks (*SI Appendix*, Fig. S4 *E* and *F*, respectively). Furthermore, we could also detect significantly elevated levels of the inflammatory cytokines BLC, IL-1α, TNF-α and IL-1β with black ink tattooing compared to PBS-tattooed control groups (Fig. 4*F*). Finally, to evaluate whether the presence of the ink might be associated with higher cell proliferation in the draining LNs of tattooed patients, we performed immunohistology staining with KI-67, which demonstrated areas of cell proliferation in a LN associated with the area where ink accumulates, compared to areas without pigment (*SI Appendix*, Fig. S4*G*).

Tattoo Ink Alters the Immune Response to Vaccination. To study the short- and long-term effects of tattooing on the antibody response following vaccination, we administered the Pfizer-BioNTech COVID-19 vaccine to mice at two different time points (2 d or 2 mo) after being tattooed with black, red, or green ink, and we evaluated the Ig responses against the receptor binding domain (RBD) of the spike protein. We found that all tattooed groups showed a significant decrease in the levels of anti-RBD specific IgG at 10 d postvaccination (p.v.) (Fig. 5*A*). In contrast, only the animals tattooed with red and green ink showed significantly decreased levels of IgM at 7 d p.v. (*SI Appendix*, Fig. S5*A*). Notably, the deficiency in the anti-RBD specific IgG responses was maintained in all the groups that were tattooed 2 mo before (Fig. 5*B*). However, we could not observe significant differences in the IgM response (*SI Appendix*, Fig. S5*B*). To evaluate whether the presence of the ink could affect the expression of the coronavirus spike protein in the MM, we performed immunostaining with an anti-spike antibody followed by FACS analysis at 24 h p.v. in two groups of mice tattooed 48 h or 2 mo before. We observed that MM from all tattooed groups showed a tendency to display reduced levels of expression of the spike protein in the short-term experiments (Fig. 5*B*). Furthermore, at 2 mo p.t., the expression levels of the spike protein were significantly decreased in MM from all the tattooed groups (Fig. 5*D*), compared to the control groups. Additionally, we observed a significant decrease in the expression of the costimulatory markers CD86 and CD80 in MM at 48 h and 2 mo p.t, respectively. Specifically, CD86 levels were reduced in MM across all tattooed groups at 48 h p.t. (Fig. 5 *E*, *Left*), while CD80 levels decreased in MM of the black and green ink tattooed groups at 2 mo p.t. (Fig. 5 *F*, *Middle*). Conversely, even if the costimulatory marker MHC-II tended to increase in all tattooed groups at 48 h p.t., the level declined by 2 mo p.t (Fig. 5 *E* and *F*, *Right*). To confirm that decreased antibody response was due to an ink-dependent impairment of the LN antigen-presenting cells (APCs), we tattooed one footpad of the mice with black, red, or green ink, and we administered the Pfizer-BioNTech COVID-19 vaccine in the contralateral one 48 h p.t. As expected, the anti-RBD specific IgG response was maintained when the vaccine was administered in the contralateral footpad (*SI Appendix*, Fig. S5*C*).

To evaluate whether the presence of ink could also affect the immune response in human cells, we differentiated macrophages from PBMCs of six healthy donors, added different amounts of the three tested inks together with the Pfizer-BioNTech COVID-19 vaccine, and coculture them with B and T cells from the same donors. Interestingly, we observed that the presence of all inks significantly reduced the anti-spike-specific IgG produced by B cells at day nine p.v. with the tested concentrations of ink (*SI Appendix*, Fig. S5*D*). However, as in the case of the mouse, the levels of IgM produced were only significantly reduced after treatment with the red and green inks (*SI Appendix*, Fig. S5*E*). Furthermore, as demonstrated in the murine model, the presence of black ink in the human macrophages significantly reduced the capture of the labeled vaccine (*SI Appendix*, Fig. S5*F*), as well as the expression of the spike protein in these cells (*SI Appendix*, Fig. S5*G*).

While mRNA vaccines, as the Pfizer-BioNTech COVID-19, require the uptake by host cells to produce the target antigen and initiate an immune response (25), this mechanism of action is not true for other kind of vaccines. For this reason, we evaluated the short- and long-term effects of tattooing on the anti-influenza antibody response following vaccination with UV-inactivated A/Puerto Rico/8/34 strain virus (H1N1). We have previously characterized the relevance of the inflammatory response for the development of an effective humoral response in this vaccine model (26, 27). Notably, we found that black and red ink showed a significant increase in the levels of anti-influenza specific IgM at 7 d p.v., while only the red group showed increased anti-influenza specific IgG at

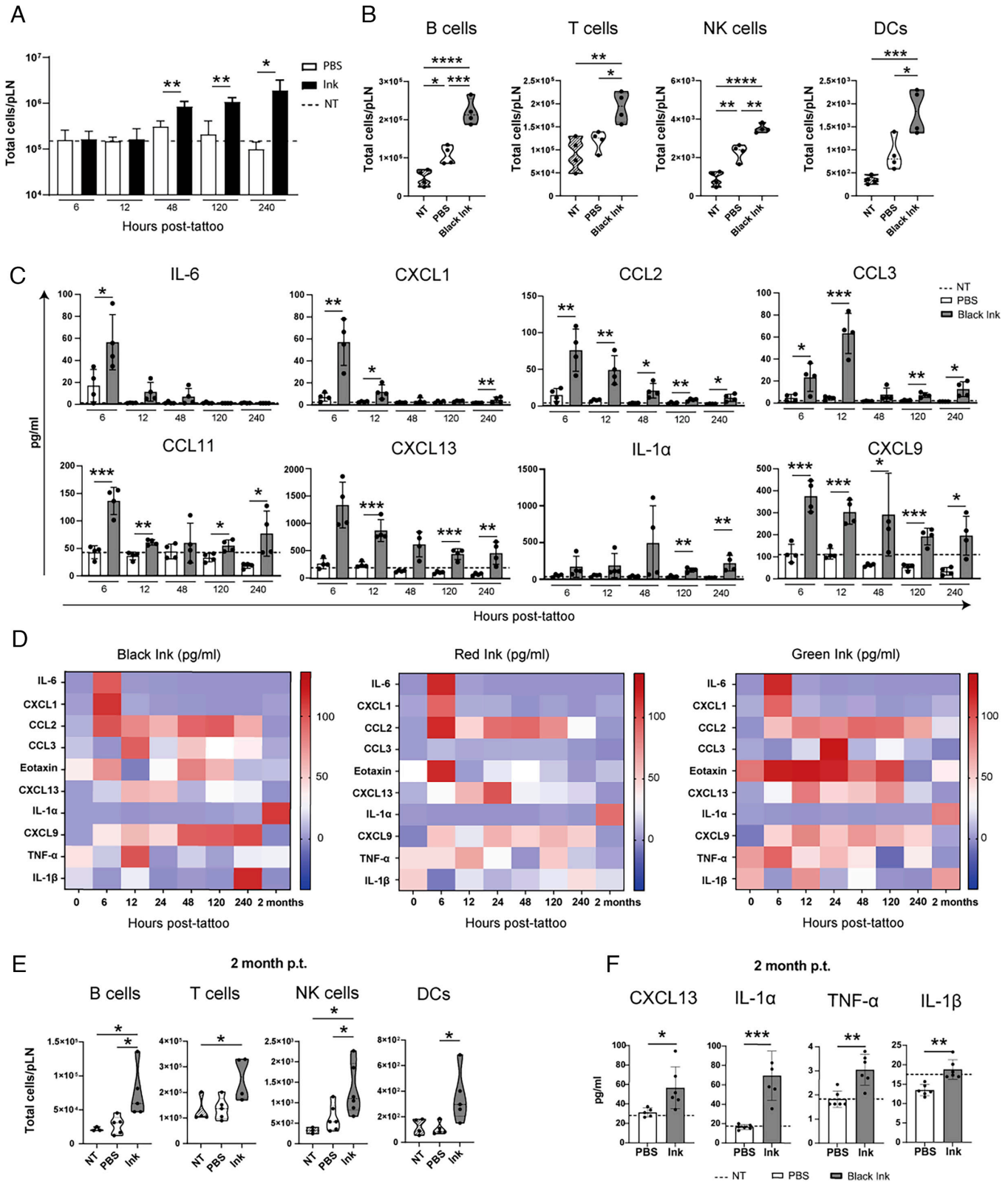

Fig. 4. Characterization of the inflammatory response induced by tattoo ink in the lymphatic compartment. (*A*) Time course showing the total number of cells in the pLN during the first 240 h p.t. (*B*) Total number of B, T, NK cells, and DCs in the p LN at 48 h p.t. (*C*) Cytokine and chemokine levels in the draining LN during the 240 h that follows tattooing with black ink. Heatmap showing the expression of different cytokines and chemokines in the blood at various time points following tattooing with black (*Left*), red (*Middle*), and green ink (*Right*). (*E*) Total number of B, T, NK cells, and DCs in the pLN at 2 mo p.t. (*F*) Expression of CXCL13, IL-1α, TNF-α, and IL-1β, in the pLN at 2 mo following black ink tattooing. In (*A*–*F*), one out of two independent experiments is shown. Popliteal lymph node (pLN). Data are presented as mean ± SD. One-way (*B* and *E*) or Two-way ANOVA (*A* and *C*) followed by Bonferroni correction for multiple comparisons (*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, and ****$P < 0.0001$).

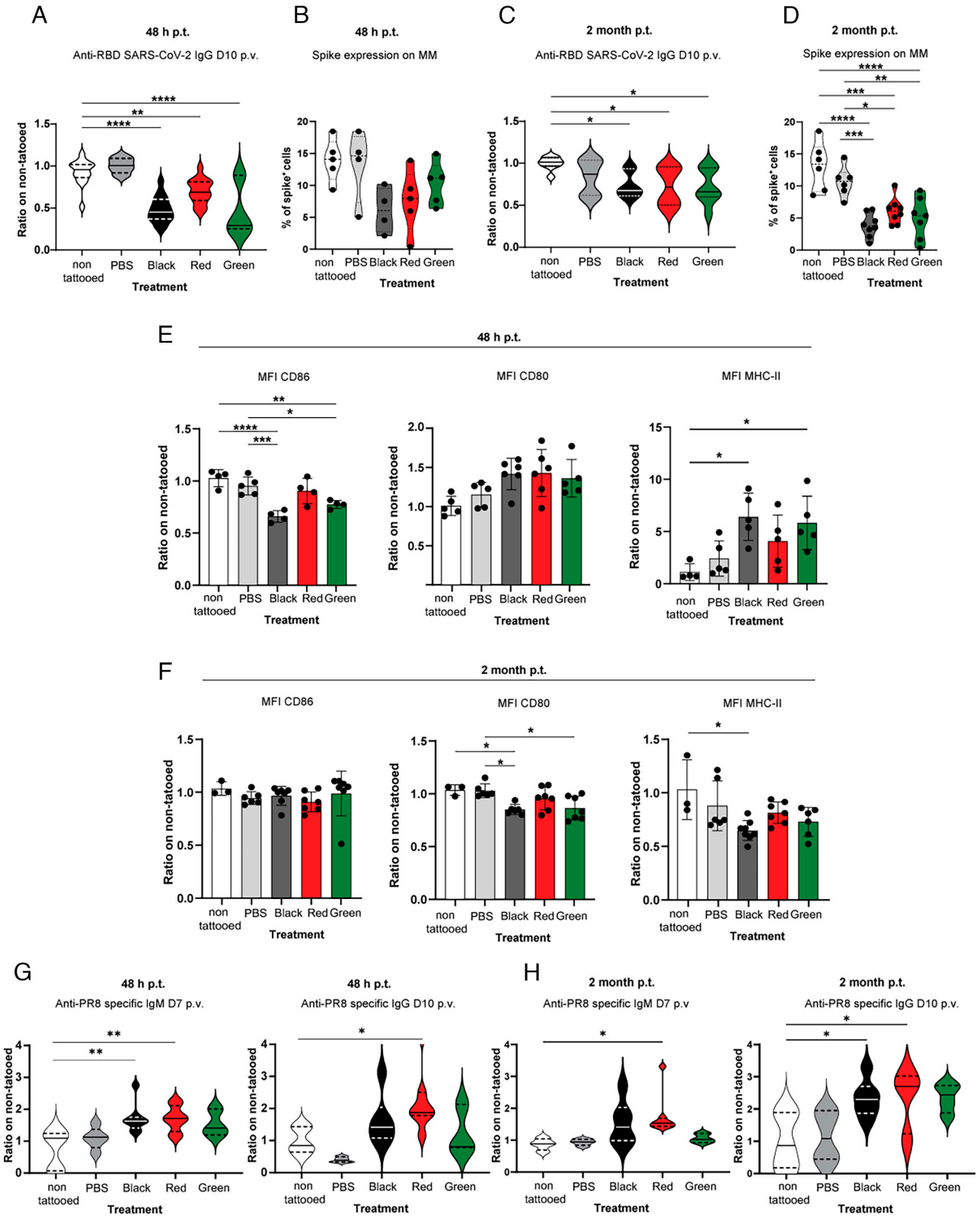

Fig. 5. Characterization of the immune response to vaccines after short- and long-term tattooing. (*A*) Violin plot showing the anti-RDB SARS-CoV-2 IgG titers at day 10 p.v. in animals tattooed 48 h before. n = 14 for the nontattooed group, n = 5 for the PBS group, n = 15 for black and red groups, and n = 7 for the green group. (*B*) Expression of coronavirus spike protein on MM at 24 h p.v. in animals tattooed 48 h before. (*C*) Violin plot of the anti-RDB SARS-CoV-2 IgG titers at day 10 p.v. in long-term (2 mo) tattooed animals. n = 8 for each group. (*D*) Expression of coronavirus spike protein at day 10 p.v. in MM in a 2-mo posttattooing (p.t.) group. (*E*) Quantification of the expression of the costimulatory molecules CD86, CD80, and MHC-II in MM at 24 h p.v. in animals tattooed 48 h or 2 mo (*F*) before vaccination. (*G*) Violin plot showing the anti-PR8 IgGM and IgG titers at day 7 and 10 p.v. in animals tattooed 48 h before. n = 5 for the nontattooed group, n = 5 for the PBS group, n = 8 for black and red groups, and n = 5 for the green group. (*H*) Violin plot showing the anti-PR8 IgGM and IgG titers at day 7 and 10 p.v. in animals tattooed 2 mo before. n = 5 for the nontattooed group, n = 7 for the PBS group and black and red groups, and n = 5 for the green group. In all graphs, the ratio represents the fold on the vaccinated nontattooed group. Medullary macrophage (MM). Data are presented as median and 25th (*Bottom*) and 75th (*Top*) percentiles or mean ± SD. One-way ANOVA followed by Bonferroni correction for multiple comparisons (*$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$).

10 d postvaccination (Fig. 5*G*). In contrast, among the mice tattooed 2 mo before only the animals tattooed with red ink showed significantly increased levels of IgM at 7 d p.v., while both black and red inks displayed elevated levels of IgG at 10 d p.v. (Fig. 5*H*).

Discussion

One of the urgent concerns associated with the safety of tattoos regards the potential redistribution of the unretained ink from the tattoo site to organs other than the skin and the toxic effect that the accumulation of these insoluble pigments might have at systemic levels. Previous studies have reported the deposition of pigments in the draining lymph node (dLN) (28–36). Nevertheless, the contribution that the lymphatic vessels and bloodstream might play in this process needs to be further studied. Our work suggests that most unretained ink from the skin disseminates via the lymphatic system and accumulates in the medullary part of the dLN in the initial instances following tattooing. Moreover, we reported a progressive increase of ink pigment observed in the dLN 2 mo posttattoo, probably associated with constant draining from the tattooed site at the skin. This is particularly relevant considering that the puncturing of dermal blood vessels during the tattooing process in humans might also contribute to disseminating the ink via the bloodstream. Indeed, some studies have reported the presence of tattoo ink systemically associated with Kupffer cells of the liver, indicating that a blood-borne distribution of tattoo ink might also occur (37). In addition, the extensive tattooed areas pose an added risk for the systemic distribution of the ink. Previous studies have estimated that on average, 2.5 mg/cm^2 is introduced into the dermal layer during the tattooing process (13, 33). Therefore, further studies are needed to evaluate, especially in people who get large tattoos, the potential correlation between the size of the tattoo and the accumulation of ink in internal organs, such as the spleen, liver, and kidney, as well as its pathophysiological consequences.

Although the adverse effects of tattoo pigment on the skin have been previously reported (38), the impact of the accumulated pigments on the immune response is still unknown. In this work, we have demonstrated that macrophages exposed to different ink concentrations undergo apoptotic cell death with all the tested inks. A previous study has pointed out that monocytes and macrophages are the main immune cells involved in ink particle uptake after tattooing. It has been demonstrated that monocytes undergo significant alterations during their differentiation into macrophages, which could influence their viability and their sensitivity to tattoo ink (39). The interaction of ink pigment with macrophages depends on their physicochemical characteristics and can lead to cytotoxic events, including cell viability perturbation via apoptosis or necrosis, oxidative stress, and reactive oxygen species (ROS) generation (40, 41). Furthermore, we have demonstrated that the ink pigments retained in the medullary region of the dLN stay associated with the phagocytic populations for months following tattooing. This is in line with other human studies in which ink pigments in dLNs were detected years after tattooing (34). LN macrophages constantly filter the lymph and are essential for initiating innate immune responses that lead to the capture and inactivation of lymph-borne pathogens (26, 42–44). The accumulation of ink pigments, especially in the medullary region, is particularly relevant due to the highly phagocytic ability of these cells, specialized in the clearance of particulates, pathogens, and dying cells from the lymph and in support of plasma cell survival (45). Considering the critical role of these cells in antimicrobial immunity, we hypothesize that tattoo ink pigment accumulation and the observed induction of macrophage death by the different types of ink could affect the capacity of these cells to control the spreading of pathogenic viruses and bacteria via the lymph, increasing the risk of dissemination. In this direction, previous studies have demonstrated that LN macrophages play a critical role in capturing lymph-borne viruses such as vesicular stomatitis virus (VSV), adenovirus, vaccinia virus, and murine cytomegalovirus (46). Additionally, the depletion of LN macrophages before the VSV challenge led to a significant reduction in survival and an increase in viral titers found in the brain and spinal cord of the depleted mice (47). The effect of the presence of ink in these cells on their antimicrobial function needs to be further evaluated in future studies.

The activation of the innate immune compartment is most likely associated with the prominent inflammatory response described in this study. This reaction in the lymphatic compartment could also be linked with the well-characterized acute inflammation caused by needles in the skin (48), which might increase the chances of a dysregulated inflammatory response in patients who have developed allergic reactions or autoimmune diseases. Despite a common inflammatory trend observed in all tested pigments, significant differences were also associated with the capacity of certain pigments to induce inflammatory mediators. The initial acute inflammatory response was probably influenced, both at the cellular and molecular level, not only by the pigment alone but also by the presence of other ink components, such as binders, solvents, and additives known to be toxic (4). These differences stressed the need for a careful evaluation of the safety profile of each of the chemical components of the tattoo ink mixture and the implementation of regulations that can reduce the heterogeneity in inks produced by different companies.

Additionally, different studies have previously associated chronic inflammation with multiple pathologies, including cancer (49). In this direction, a recent study has confirmed the association between tattoo exposure and an increased risk of malignant lymphoma (50). Furthermore, the elevated levels of the alarmin IL-1α in the draining LN were maintained during the first 2 mo following tattooing with black ink. Interestingly, we have previously associated the elevated levels of this cytokine with the initiation of melanoma metastasis in the LN (51). In addition, the observed chronic inflammation could also affect the immune surveillance and response to therapy in some tattooed cancer patients who undergo immune treatment (52). A recent study analyzed the usage of checkpoint BRAF/MEK inhibitors in tattooed patients with melanoma and the consequent adverse granulomatous reaction in the skin, which may be due to the enhanced loss of tolerance to tattoo inks (53). Finally, the chronic inflammatory setting could potentially increase the carcinogenicity associated with certain pigments or their byproducts, increasing the risk of developing neoplasia (54). Further studies are needed to identify the molecular basis of the connection between tattooing and cancer.

During the COVID-19 pandemic, one of the general concerns of the practitioners was the influence that administering a vaccine to a tattooed individual might have on developing the antibody response against the virus (55). To date, no epidemiological studies have evaluated how the presence of a tattoo may affect the COVID-19 vaccine efficiency. In this work, we demonstrated that the presence of tattoo ink crystals, at the site of vaccine administration, can modulate the immune response in a vaccine-specific manner. We showed the negative effect that the accumulation of ink has on the expression of the spike protein and, ultimately, in the humoral immune response elicited by the vaccine. Moreover, especially with red and black inks, we observed that the response to a UV-inactivated influenza vaccine was enhanced. This suggests that tattoo-induced inflammation and the presence of ink pigment

particles at the site of tattoo injection may interact differently with various vaccine formulations, whose mechanisms of action may differ, potentially influencing antigen presentation, local inflammation, or innate immune activation. The alteration in the immune response to vaccines in the animals tattooed with black ink may be associated with the capacity of carbon black to interfere with the antigen-processing mechanism in lymphocytic populations. Conversely, tattoo-induced local inflammation can act as an adjuvant-like stimulus, enhancing vaccine responses, similar to aluminum salts activating the NLRP3 inflammasome (56, 57). This effect, also seen in tattoo-mediated DNA vaccination (58), may improve the efficacy of inactivated vaccines such as influenza vaccine.

Our results need to be further validated in a human study. However, different parameters need to be considered, such as the size and position of the tattoo and the type of ink used by the study cohort. Moreover, further studies assessing the effect of tattooing on the immune response induced by different vaccine types or for several diseases are needed to elucidate whether the effect found with the tested vaccines can be generalized to other classes. Tattooing might be an added risk for those individuals vaccinated with attenuated vaccines. Some reports associated with the live smallpox vaccination program in military service members have reported smallpox complications in different vaccinated, tattooed patients (59). Future studies are needed to characterize these responses in more detail.

The toxicology risk of the components formed by tattoo inks remains a matter of concern (60). A tattoo ink consists of a multicomponent mixture of solvents, preservatives, additives, and nonliquid components like insoluble solid-state pigment particles. These pigments are responsible for the color of the tattoo. However, being a complex mixture of chemicals, different ink colors and suppliers contain different ingredients and impurities that may be responsible for the specific and heterogeneous cellular toxicity described. Further analyses are needed to investigate the mechanisms by which such a complex mixture is transported through the lymphatic system. This includes identifying which chemical components travel with the lymph and, together with insoluble pigments, contribute to the observed effects. The lack of regulation of the chemical component of the tattoo ink mixture is critical for this kind of analysis in which inks from different suppliers show a specific blend of ingredients with varying toxicity profiles. This study evaluated the three most used commercial tattoo ink colours: black (50%), red (14%), and green (9.1%) (61). The observed differences in the toxicity effect of the tested inks are consistent with previous studies that identify red dyes as the most problematic, often associated with a cutaneous inflammatory response (62, 63). Red and black pigments are characterized by an aggregation of particles over time, resulting in larger pigment foreign bodies. This aggregation of pigments can be critical in developing local and systemic tattoo complications and can affect cellular uptake and transportation via the lymphatic system (64).

In summary, this work represents the most extensive study to date regarding the effect of tattoo ink on the immune response and raises serious health concerns associated with the tattooing practice. Our work underscores the need for further research to inform public health policies and regulatory frameworks regarding the safety of tattoo inks.

Material and Methods

Full descriptions of the experimental methods and reagents can be found in *SI Appendix*.

Study Design and Approval. This study aimed to characterize immune responses to tattoo ink accumulating in draining LNs and to determine how its presence modulates vaccine response. We used C57BL/6 J, CX3CR1-GFP, and CD11c-YFP mice, bred under SPF conditions, to examine the distribution and immune effects of tattoo inks following footpad tattooing. Mice (6 to 12 wk) were tattooed with commercial inks or PBS control. In selected experiments, BNT162b2 mRNA vaccines or UV-inactivated influenza A virus strain A/Puerto Rico/8/1934 (H1N1) were injected into tattooed footpads to assess local effects on vaccine capture and response. Blood and draining (popliteal and lumbar) LNs were collected at defined time points for integrated analyses, including two-photon intravital microscopy, immunofluorescence, EM, flow cytometry, and multiplex cytokine profiling. Chromatographic methods analyzed the ink composition. To assess the uptake of ink by immune cells and their subsequent viability, we performed in vitro studies using murine bone marrow–derived and human monocyte–derived macrophages cocultured with the three different inks. All animal experiments complied with Swiss Federal Veterinary Service regulations and were approved (permit TI 58/2021 and TI 32/2025) by the Cantonal Animal Welfare Committee. Human blood samples were obtained with informed consent and approval from the Ethical Committee of Canton Ticino (CD-3428). A comprehensive description of the experimental procedures and reagents is provided in *SI Appendix*.

Data, Materials, and Software Availability. All study data are included in the article and/or *SI Appendix*.

ACKNOWLEDGMENTS. This work was supported by Swiss NSF grant 204636 (C.P., L.R., K.C., and S.F.G.), Swiss Centre for Applied Human Toxicology (SCAHT) (to A.C.) funding project 2022-2024, Swiss Government Excellence Scholarship (to J.F.), Fidinam Foundation, and the Horten Foundation.

Author affiliations: [a]Infection and Immunity Laboratory, Faculty of Biomedical Sciences, Institute for Research in Biomedicine, Università della Svizzera italiana, Bellinzona 6500, Switzerland; [b]Charité – Universitätsmedizin Berlin, corporate member of Freie Universität Berlin and Humboldt-Universität zu Berlin, Institute of Physiology, Berlin 10117, Germany; [c]Graduate School for Cellular and Biomedical Sciences, University of Bern, Bern 3012, Switzerland; [d]Ente Ospedaliero Cantonale, Istituto Cantonale di Patologia, Locarno 6600, Switzerland; [e]Department of Dermatology, Allergology and Venereology, Hannover Medical School, Hannover 30625, Germany; [f]Basel Kantonal Laboratory, Chromatographie Group, Basel 4056, Switzerland; [g]Protein Folding and Quality Control, Faculty of Biomedical Sciences, Institute for Research in Biomedicine, Università della Svizzera italiana, Bellinzona 6500, Switzerland; [h]Faculty of Biomedical Sciences, Institute of Oncology Research, Università della Svizzera Italiana, Bellinzona 6500, Switzerland; [i]Department of Infectious Diseases and Pathobiology, Institute of Animal Pathology, Comparative Pathology Platform, Vetsuisse Faculty, University of Bern, Bern 3012, Switzerland; [j]Department of Dermatology, University Hospital Regensburg, Regensburg 93053, Germany; [k]Institute of Parasitology, Biology Centre of the Czech Academy of Sciences, Ceske Budejovice 370 05, Czech Republic; [l]Veterinary Research Institute, Brno 621 00, Czech Republic; [m]Institute of Experimental Biology, Faculty of Science, Masaryk University, Brno 602 00, Czech Republic; [n]World Health Organization Classification of Tumours, International Agency for Research on Cancer, Lyon 69372, France; and [o] Environment and Lifestyle Epidemiology Branch, International Agency for Research on Cancer, Lyon 69372, France

Author contributions: A.C., J.F., and S.F.G. designed research; A.C., J.F., C.P., I.L., L.M., V.S., A.R., T.V., A.P., S.M., L.G.-R., W.B., M.P., L.R., M.F., K.C., and S.F.G. performed research; U.H., W.B., D.R., and G.G.L. contributed new reagents/analytic tools; S.F.G. analyzed data; and A.C., J.F., and S.F.G. wrote the paper.

1. N. Kluger, S. Seité, C. Taieb, The prevalence of tattooing and motivations in five major countries over the world. *J. Eur. Acad. Dermatol. Venereol.* **33**, e484–e486 (2019), 10.1111/jdv.15808.
2. N. Kluger, Epidemiology of tattoos in industrialized countries. *Curr. Probl. Dermatol.* **48**, 6–20 (2015), 10.1159/000369175.
3. K. Schaeffer, S. Dinesh, 32% of Americans have a tattoo, including 22% who have more than one. *Pew Research Center* (2023).
4. M. Dirks, Making innovative tattoo ink products with improved safety: Possible and impossible ingredients in practical usage. *Curr. Probl. Dermatol.* **48**, 118–127 (2015).
5. M. Giulbudagian *et al.*, Lessons learned in a decade: Medical-toxicological view of tattooing. *J. Eur. Acad. Dermatol. Venereol.*, 1926–1938 (2024), 10.1111/jdv.20072.
6. J. Serup, N. Kluger, W. Bäumler, Tattooed Skin and Health, Karger Medical and Scientific Publishers, 26 March 2015.
7. W. Bäumler *et al.*, Q-switch laser and tattoo pigments: First results of the chemical and photophysical analysis of 41 compounds. *Lasers Surg. Med.* **26**, 13–21 (2000).
8. Kitchen hygiene in the spotlight food supplements in sports, German Federal Institute for Risk Assessment, ISBN 978-3-943963-85-4, www.bfr.bund.de/en.

9. A. Maria, B. Rico, EU regulation on substances in tattoo inks and permanent make-up chances and challenges, Bundesinstitut für Risikobewertung (2021).
10. A. Baranska *et al.*, Unveiling skin macrophage dynamics explains both tattoo persistence and strenuous removal. *J. Exp. Med.* **215**, 1115–1133 (2018).
11. T. Paul Litton, S. Vijay Ghate, Tattoo pigment mimicking axillary lymph node calcifications on mammography. *Radiol. Case Rep.* **15**, 1194–1196 (2020).
12. C. M. Jack, A. Adwani, H. Krishnan, Tattoo pigment in an axillary lymph node simulating metastic malignant melanoma. *Int. Semin. Surg. Oncol.* **2**, 1–3 (2005).
13. W. Bäumler, Absorption, distribution, metabolism and excretion of tattoo colorants and ingredients in mouse and man: The known and the unknown. *Curr. Probl. Dermatol.* **48**, 176–184 (2015).
14. S. L. Peterson, L. A. Lee, K. Ozer, J. E. Fitzpatrick, Tattoo pigment interpreted as lymph node metastasis in a case of subungual melanoma. *Hand* **3**, 282–285 (2008).
15. I. Balasubramanian, J. P. Burke, E. Condon, Painful, pigmented lymphadenopathy secondary to decorative tattooing. *Am. J. Emerg. Med.* **31**, 1001.e1–1001.e2 (2013).
16. J. Serup, H. C. Katrina, Patch test study of 90 patients with tattoo reactions: Negative outcome of allergy patch test to baseline batteries and culprit inks suggests allergen(s) are generated in the skin through haptenization. *Contact Dermatitis* **71**, 255–263 (2014).
17. E. Forbat, F. Al-Niaimi, Patterns of reactions to red pigment tattoo and treatment methods. *Dermatol. Ther.* **6**, 13–23 (2016), 10.1007/s13555-016-0104-y.
18. N. V. Gopee *et al.*, Response of mouse skin to tattooing: Use of SKH-1 mice as a surrogate model for human tattooing. *Toxicol. Appl. Pharmacol.* **209**, 145–158 (2005).
19. E. Engel *et al.*, Tattooing of skin results in transportation and light-induced decomposition of tattoo pigments–A first quantification in vivo using a mouse model. *Exp. Dermatol.* **19**, 54–60 (2010).
20. N. Kluger, Is it safe to vaccinate within a tattoo? *Ann. Dermatol. Venereol.* **148**, 256–258 (2021), 10.1016/j.annder.2021.04.008.
21. Commission Regulation (EU) 2020/2081 of 14 December 2020 amending annex XVII to regulation (EC) No 1907/2006 of the European parliament and of the council concerning the registration, evaluation, authorisation and restriction of chemicals (REACH) as regards substances in tattoo inks or permanent make-up. *Official Journal of the European Union*, L 423/6–423/17 (2020). https://eur-lex.europa.eu/eli/reg/2020/2081/oj/eng.
22. G. Arroyo-Ataz, A. C. Yagüe, J. C. Breda, S. A. Mazzilli, D. Jones, Transcriptional, developmental, and functional parallels of lymphatic and venous smooth muscle. bioRxiv [Preprint] (2024). https://www.biorxiv.org/content/10.1101/2024.07.18.604042v1 (Accessed 22 July 2024).
23. N. I. Walker, B. V. Harmon, G. C. Gobe, J. F. R. Kerr, Patterns of cell death. *Methods Achiev. Exp. Pathol.* **13**, 18–54 (1988).
24. F. J. Bock, J. S. Riley, When cell death goes wrong: Inflammatory outcomes of failed apoptosis and mitotic cell death. *Cell Death Different.* **30**, 293–303 (2023), 10.1038/s41418-022-01082-0.
25. N. Pardi, M. J. Hogan, F. W. Porter, D. Weissman, mRNA vaccines–A new era in vaccinology. *Nat. Rev. Drug Discov.* **17**, 261–279 (2018), 10.1038/nrd.2017.243.
26. N. Chatziandreou *et al.*, Macrophage death following influenza vaccination initiates the inflammatory response that promotes dendritic cell function in the draining lymph node. *Cell Rep.* **18**, 2427–2440 (2017).
27. Y. Farsakoglu *et al.*, Influenza vaccination induces NK-cell-mediated type-II IFN response that regulates humoral immunity in an IL-6-dependent manner. *Cell Rep.* **26**, 2307–2315.e5 (2019).
28. C. M. Lerche, M. Sepehri, J. Serup, T. Poulsen, H. C. Wulf, Black tattoos protect against UVR-induced skin cancer in mice. *Photodermatol. Photoimmunol. Photomed.* **31**, 261–268 (2015).
29. E. Dominguez *et al.*, Tattoo pigment in two lymph nodes in a patient with melanoma. *J. Eur. Acad. Dermatol. Venereol.* **22**, 101–102 (2008), 10.1111/j.1468-3083.2007.02112.x.
30. A. Schlager, A. Laser, J. Melamed, A. A. Guth, A tattoo-pigmented node masquerading as the sentinel node in a case of breast cancer. *Breast J.* **14**, 299–300 (2008).
31. T. Friedman, M. Westreich, S. N. Mozes, A. Dorenbaum, O. Herman, Tattoo pigment in lymph nodes mimicking metastatic malignant melanoma. *Plast. Reconstr. Surg.* **111**, 2120–2122 (2003).
32. M. Moehrle, H. J. Blaheta, P. Ruck, Tattoo pigment mimics positive sentinel lymph node in melanoma. *Karger. Dermatology.* 203, 342–344 (2001). www.karger.comwww.karger.com/journals/drm.
33. E. Engel *et al.*, Modern tattoos cause high concentrations of hazardous pigments in skin. *Contact Dermatitis* **58**, 228–233 (2008).
34. K. Lehner *et al.*, Black tattoos entail substantial uptake of genotoxicpolycyclic aromatic hydrocarbons (PAH) in human skin and regional lymph nodes. *PLoS One* **9**, e92787 (2014).
35. S. Chikkamuniyappa, R. Sjuve-Scott, K. Lancaster-Weiss, A. Miller, I.-T. Yeh, Tattoo pigment in sentinel lymph nodes: A mimicker of metastatic malignant melanoma. *Dermatol. Online J.* **11** (2005).
36. L. L. Anderson, J. S. Cardone, M. L. McCollough, W. J. Grabski, Tattoo pigment mimicking metastatic malignant melanoma. *Dermatol. Surg.* **22**, 92–94 (1996).
37. M. Sepehri, T. Sejersen, K. Qvortrup, C. M. Lerche, J. Serup, Tattoo pigments are observed in the Kupffer cells of the liver indicating blood-borne distribution of tattoo ink. *Dermatology* **233**, 86–93 (2017).
38. S. Schubert, N. Kluger, I. Schreiver, Hypersensitivity to permanent tattoos: Literature summary and comprehensive review of patch tested tattoo patients 1997–2022. *Contact Dermatitis* **88**, 331–350 (2023), 10.1111/cod.14291.
39. C. Lin *et al.*, Macrophage-like rapid uptake and toxicity of tattoo ink in human monocytes. *Immunology* **171**, 388–401 (2024).
40. Y. Niu, M. Tang, In vitro review of nanoparticles attacking macrophages: Interaction and cell death. *Life Sci.* **307**, 1–11 (2022), 10.1016/j.lfs.2022.120840.
41. M. Awashra, P. Młynarz, The toxicity of nanoparticles and their interaction with cells: An in vitro metabolomic perspective. *Nanoscale Adv.* **5**, 2674–2723 (2023), 10.1039/d2na00534d.
42. S. Gordon, F. O. Martinez, Alternative activation of macrophages: Mechanism and functions. *Immunity* **32**, 593–604 (2010), 10.1016/j.immuni.2010.05.007.
43. I. Moran, A. K. Grootveld, A. Nguyen, T. G. Phan, Subcapsular sinus macrophages: The seat of innate and adaptive memory in murine lymph nodes. *Trends Immunol.* **40**, 35–48 (2019), 10.1016/j.it.2018.11.004.
44. M. Kuka, M. Iannacone, The role of lymph node sinus macrophages in host defense. *Ann. N. Y. Acad. Sci.* **1319**, 38–46 (2014).
45. E. E. Gray, J. G. Cyster, Lymph node macrophages. *J. Innate Immunity* **4**, 424–436 (2012), 10.1159/000337007.
46. D. A. P. Louie, S. Liao, Lymp node subcapsular sinus macrophages as the frontline of lymphatic immune defense. *Front. Immunol.* **10**, 1–9 (2019).
47. M. Iannacone *et al.*, Subcapsular sinus macrophages prevent CNS invasion on peripheral infection with a neurotropic virus. *Nature* **465**, 1079–1083 (2010).
48. A. Bassi *et al.*, Tattoo-associated skin reaction: The importance of an early diagnosis and proper treatment. *Biomed. Res. Int.* 2014, 1–7 (2014).
49. A. Mantovani, P. Allavena, A. Sica, F. Balkwill, Cancer-related inflammation. *Nature* **454**, 436–444 (2008), 10.1038/nature07205.
50. C. Nielsen, M. Jerkeman, A. S. Jöud, Tattoos as a risk factor for malignant lymphoma: A population-based case–control study. *EClinicalMedicine* **72**, 102649 (2024).
51. T. Virgilio *et al.*, Subcapsular sinus macrophages promote melanoma metastasis to the sentinel lymph nodes via an IL1a–STAT3 axis. *Cancer Immunol. Res.* **10**, 1525–1541 (2022).
52. S. I. Grivennikov, F. R. Greten, M. Karin, Immunity, Inflammation, and Cancer. *Cell* **140**, 883–899 (2010), 10.1016/j.cell.2010.01.025.
53. N. A. Isaq *et al.*, Granulomatous tattoo reaction associated with BRAF/MEK inhibitor therapy for metastatic melanoma. *Int. J. Dermatol.*, 1630–1632 (2024), 10.1111/ijd.17273.
54. M. R. Galdiero, G. Marone, A. Mantovani, Cancer inflammation and cytokines. *Cold Spring Harb. Perspect. Biol.* **10**, a028662 (2018).
55. M. A. Kluger *et al.*, Convalescent plasma treatment for early post-kidney transplant acquired COVID-19. *Transpl. Infect. Dis.* **23**, 1–3 (2021), 10.1111/tid.13685.
56. L. Franchi, G. Núñez, The Nlrp3 inflammasome is critical for aluminum hydroxide-mediated IL-1β secretion but dispensable for adjuvant activity. *Eur. J. Immunol.* **38**, 2085–2089 (2008).
57. M. B. Kool, H. van Middendorp, M. A. Lumley, J. W. J. Bijlsma, R. Geenen, Social support and invalidation by others contribute uniquely to the understanding of physical and mental health of patients with rheumatic diseases. *J. Health Psychol.* **18**, 86–95 (2013).
58. D. Pokorna, I. Rubio, M. Müller, DNA-vaccination via tattooing induces stronger humoral and cellular immune responses than intramuscular delivery supported by molecular adjuvants. *Genet. Vaccines Ther.* **6**, 4 (2008).
59. B. M. Carius, P. M. Dodge, J. D. Randles, Smallpox autoinoculation via tattoo in a soldier. *Mil. Med.* **184**, E275–E279 (2019).
60. P. Laux *et al.*, A medical-toxicological view of tattooing. *Lancet* **387**, 395–402 (2016), 10.1016/S0140-6736(15)60215-X.
61. I. Klügl, K. A. Hiller, M. Landthaler, W. Bäumler, Incidence of health problems associated with tattooed skin: A nation-wide survey in German-speaking countries. *Dermatology* **221**, 43–50 (2010).
62. J. M. Sowden *et al.*, Red tattoo reactions. *Br. J. Dermatol.* **124**, 576–580 (1991).
63. G. Chapman, C. Anne, T. Hildyard, Two decades later: A delayed red ink tattoo reaction. *BMJ Case Rep.* **2014**, bcr2013201726 (2014), 10.1136/bcr-2013.
64. K. Hutton Carlsen, G. Larsen, J. Serup, Tattoo pigment agglomerates measured in commercial ink stock products by computerised light microscopy. *Skin Res. Technol.* **26**, 292–300 (2020).